REMARKS

Applicant respectfully requests a three month extension of time to respond to the May 5, 2008 Office Action. Filed herewith is a Request for Extension of Time Pursuant to 37 CFR 1.136a and the requisite fee for this extension is attached hereto.

Status of the claims:

The Office Action Summary of May 5, 2008 reports under Disposition of Claims that claims 1-4, 7-12, and 16-25, 32, 33, 38 and 40 are pending in the application. As a correction, Applicant wishes to point out that in the response filed on May 01, 2008 claims 5-6, 26-33, 38, and 40 were canceled and that claims 13-15, 34-37, 39 and 41-63 were previously canceled. Thus, claims 1-4, 7-12 and 16-25 are pending and ready for further action on the merits.

Claims 1-4, 7-12, 16-25, 32, 33 and 40 stand rejected under 35 USC 112, first paragraph.

In a separate rejection, claims 32, 33, and 40 also stand rejected, and claim 33 stands rejected, under 35 USC 112, first paragraph.

Rejections under 35 USC 112, first paragraph

1. Claim 1-4, 7-12, 16-25, 32, 33, 38 and 38 are rejected under 35 USC 112, first paragraph as allegedly not being enabled. Applicant notes that the rejection is moot with regard to canceled claims 32, 33, 38 and 39, and respectfully traverse the rejection with respect to claims 1-4, 7-12 and 16-25.

The Examiner asserts that the specification does not reasonably provide enablement for a solvate of a compound of formula (I).

Applicant directs the Examiner's attention to the attached decision from the Board of Patent Appeals and Interferences decision – *Ex parte Gante*, Appeal No. 2000-0600 (BPAI, 1999). Applicant respectfully points out that this decision is a non-precedential opinion. However, Applicant finds the discussion on pages 8-9 of the decision instructive. This passage states:

> *In response, appellants argue (Brief, page 3) "[t]hat all solvents cannot form solvates with all compounds is not seen to be relevant herein. The relevant inquiry is whether it would be known to one of ordinary skill in*

the art what solvents form physiologically acceptable solvates with the compounds of formula I." In this regard, appellants argue (Brief, page 4), with reference to Hybritech Inc. v. Monoclonal Antibodies, Inc., 802 F.2d 1367, 1385, 231 USPQ 81, 94 (Fed. Cir. 1986), "the selection of useful solvents and formation of solvates is highly routine in this art. Appellants need not provide in their specification a description of matter which is common and routine in the art. A 'patent need not teach, and preferably omits, what is well known in the art.'" According to appellants' (Reply Brief, page 2) "[b]ecause selection of the appropriate solvents for forming solvates was routine to one of ordinary skill in the art, the metes and bounds of the term were reasonably determinable using only ordinary skill in the art." We agree.

Applicant notes that the Board found that the formation of solvates is highly routine in the art (and confirmed *Caira's* statement discussed *infra*). The Board further said that Appellants need not provide in their specification a description of matter which is common and routine in the art (*i.e.*, forming solvates). Applicant notes that although the decision may be non-binding on the Examiner, the reasoning in the case is applicable to the present application. In addition, Applicant has set forth a detailed response to the Examiner's rejection below.

As noted by the Examiner, the factors to be considered in determining whether a disclosure meets the enablement requirement include those found in *In re Wands,* 858 F.2d 731, 737, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988), that were set forth in the Office Action of May 5, 2008 as follows:

a) the quantity of experimentation necessary,

b) the amount of direction or guidance presented,

c) the presence or absence of working examples,

d) the nature of the invention,

e) the state of the prior art,

f) the relative skill of those in the art,

g) the predictability or unpredictability of the art, and

h) the breadth of the claims.

Contrary to the Examiner's position, these *Wands* factors actually support Applicant's position that the present claims are fully enabled and therefore the Examiner's rejection of claims 1-4, 7-12 and 16-25 is respectfully traversed for this reason also.

a) the quantity of experimentation necessary

The Examiner states:

> a) Determining if a particular compound would form a solvate would require synthesis and recrystallization of the compound solvate using a variety of solvents, temperatures and humidities. The experimentation for solvates is potentially open-ended.

May 5, 2008 Office Action, page 7.

Contrary to the Examiner's position, the determination of whether a particular compound would form a polymorphic system, such as a pseudopolymorph which includes solvates and hydrates is routine in the pharmaceutical arts. *Caira* (Crystalline Polymorphism of Organic Compounds, Topics in Current Chemistry, Vol. 198 , pages 163-208, 1998) is attached. In *Caira*, examples of polymorphic systems are described which include "studies of pseudopolymorphism manifested by hydrates and solvates of the parent organic molecule". See page 164 of *Caira*. *Caira* states that "[s]ystematic investigation of a compound to determine whether it is prone to polymorphism…is routine practice in pharmaceutical pre-formulation studies." See page 165 in *Caira*. Consistent with *Caira*'s statements that "most substances when investigated for a sufficiently long time will reveal more than one polymorph" (see page 166 in *Caira*), *Caira* reviews in section 3.1 various routine preparative methods for preparing polymorphic systems including pseudopolymorphs. See pages 177 to 180 in *Caira*.

Further, even if *arguendo*, the amount of experimentation to practice the full scope of the claimed invention might be extensive that does not mean that the amount of experimentation is undue. See *Johns Hopkins Univ. v. Cellpro, Inc,* 152 F.3d 1342,1360, 47 USPQ2d 1705, 1719 (Fed. Cir 1998) ("test [for undue experimentation] is not merely quantitative…if it is merely routine").

Thus, based on the state of the art (e.g., *Caira*), and case law, this *Wands* factor supports a position that the present claims are fully enabled.

b) the amount of direction or guidance presented

The Examiner states:

> b) The specification merely mentions the Applicant's intention to make solvates, without teaching the preparation thereof.

Office Action, page 7.

As noted in the comments on a) above, the determination of whether a particular compound forms a solvate is routine in the pharmaceutical arts. Further, it is well settled that a patentee preferably omits from the disclosure any routine technology that is well

known at the time of the application. See *Hybritech, Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367,1385, (Fed.Cir. 1986).

Thus, this *Wands* factor does not support a finding that the present claims lack enablement.

c) the presence or absence of working examples

In making a determination under this factor, the Examiner cites *Morton International Inc. v Cardinal Chemical Col,* 28 USPQ2d 1190, 1194 (Fed. Cir. 1993) and then states:

> The specification shows no evidence of the formation and actual existence of solvates. Hence, Applicant must show formation of solvates or limit the claims accordingly.

Office Action, pages 7-8. Applicant respectfully disagrees.

The present specification includes a detailed description of how to make and use the compounds of the present invention, and includes over 370 examples. Thus, the specification clearly enables one of ordinary skill in the art to practice the presently claimed invention and make compounds of the present invention.

Vippagunta et. al., cited by the Examiner, at page 13 states "It has been estimated that approximately one-third of pharmaceutically active substances are capable of forming hydrates." *Vippagunta et. al.,* Advanced Drug Delivery Reviews, 48, pages 3-26, (2001). As noted in the comments on a) and b) above, "systematic investigation of a compound to determine whether it is prone to polymorphism....is routine practice in pharmaceutical pre-preparation." See page 165 in *Caira.* Thus, it would be a matter of routine practice for one of skill in the pharmaceutical arts to determine the existence of solvates of the presently claimed invention using the description provided in the present specification for making compounds of the present invention.

Further, the compound of the present claims may be contrasted with the compounds in *Morton International Inc. v. Cardinal Chemical Co.*, 28 USPQ2d 1194 (Fed. Cir. 1993) in which the disputed compounds were unidentified, unproven structures formed as a complex mixture with similar compounds of the prior art. In contrast to the compounds in *Morton International Inc.*, the solvates covered by the present claims can be formed from well defined compounds and solvents and may be identified by routine methods.

Taken as a whole, in view of the description provided in the present specification, this *Wands* factor does not support a finding that the present claims lack enablement.

d) the nature of the invention

The Examiner states:

> b) The nature of the invention is chemical synthesis of solvates, which involves chemical reactions.

Office Action, page 8.

Contrary to the Examiner's assertion, the subject matter of the present claims is not the chemical synthesis of solvates nor are the present claims directed toward a process for the preparation of solvates. The subject matter of the present claims includes compounds of formula (I) and pharmaceutically acceptable salts, solvates and prodrugs thereof.

The *Wands* factors must be examined in view of the actual subject matter of the present claims. As noted herein, the existence of solvates is well known (*Vippagunta et. al.*) and the means for forming solvates are routine (*Caira*). Therefore this *Wands* factor supports a finding that the present claims are enabled.

e) the state of the prior art

The Examiner states:

> e) The state of the art recognizes that the formation, composition and therapeutic activity of solvates in unpredictable.

Office Action, page 8. The Examiner cites *SmithKline Beecham Corp. v. Apotex Corp.* 74 USPQ2d 1398, 1409 (Fed.Cir. 2005), *Souillac et. al.*, and *Vippagunta et. al.* to support this position.

Applicant respectfully disagrees with the Examiner's position and statement.

Vippagunta et. al. presents an internally inconsistent picture on the subject of predictability and state of the art in the formation of solvates. On page 11 in section 2.5 entitled "Prediction of polymorphs", the authors report that through the use of commercial software, polymorph forms have successfully been predicted using only the molecular structure of a drug. *Vippagunta et. al.*, page 11. In discussing the state of the art, *Vippagunta* et. al. state " It has been estimated that approximately one-third of pharmaceutically active substances are capable of forming hydrates." *Id* . at page 13.

The relationship among hydrates, solvates and polymorphs is known to those of ordinary skill in the art, with hydrates and solvates sometimes being referred to as pseudopolymorphs. See, e.g., http://gow.epsrc.ac.uk/ViewGrant.aspx?GrantRef=EP/C002768/1 visited November 5, 2008 (copy attached); See also *Caira*, page 164. Thus, taken as a whole, *Vippagunta et. al.* does not support the Examiner's position. Rather, *Vippagunta et. al.* acknowledges that solvates, hydrates, polymorphs and the like are well known in the art and predictable to one of skill in the art. Further, as discussed above, *Caira* and the Board of Patent Appeals and Interferences notes that the preparation of polymorphic systems such as solvates is routine.

Further, in chemical claims, an issue related to enablement is the truth of the assertions made by the inventors. Statements made by the inventors should be consistent with generally accepted scientific principles. It is incumbent on the Patent Office to explain why it doubts the truth of any statement disclosed. *In re Marzocchi, et. al.*, 169 USPQ 367, 370 (CCPA 1971). In rejecting the presently claimed invention as lacking enablement, the Examiner has failed to show evidence or cast reasonable doubt on the truth or accuracy of any statement asserted by the applicant.

Thus, this *Wands* factor supports a finding that the present claims are enabled.

f) the relative skill of those in the art

The Examiner states:

> f) The artisan using Applicant's disclosure to prepare the claimed solvates would be, e.g., an experienced process chemist with at least a BS chemistry degree.

Office Action, page 10.

As noted in the discussion herein, one skilled in the art of pharmaceutical formulation, without regard to educational level, may through routine experimentation

prepare solvates from the currently claimed compounds. *See, e.g. Caira.* "Patents are written to enable those skilled in the art to practice the invention, not the public" *W.L. Gore Assoc., Inc v. Garlock, Inc.*, 220 USPQ 303, 315 (Fed Cir. 1983).

Thus, this *Wands* factor supports a finding that the present claims are enabled.

g) the predictability or unpredictability of the art

The Examiner states:

> g) Chemical reactions are known as unpredictable. *In re Marzocchi, et. al.*, 169 USPQ 367, 370 (CCPA 1971); *In re Fisher*, 166 USPQ 18, 24 (CCPA 1970).

Office Action, page 10.

Even if *arguendo*, the art is not capable of an absolute prediction what specific solvents will form solvates with the compounds of Formula (I) and is not capable of an absolute prediction of the specific stoichiometry of a solvate, Applicant submits that such a requirement for enablement of a claim is inconsistent with statutory standards for enablement and case law interpretation of such statutes. Predictability, by itself, is not dispositive of enablement.

For example in *In re Wands*, 858 F.2d 731, (Fed. Cir. 1988) the Federal Circuit found claims enabled where the formation of antibodies was not 100% predictable. The Federal Circuit found in Wands because the state of the art was high and the methods needed to practice the invention were well known, the claims were enabled even though the formation of any specific antibody was not predictable.

As noted herein, for example in Applicant's comments under "e) the state of the prior art", *Vippagunta et. al.* note that the formation of polymorphs, such as solvates, may be predicted using only the molecular structure of a drug. *Vippagunta et. al.*, page 11. Further, the determination of solvate formation is routine practice in the pharmaceutical arts. *See, e.g. Caira.*

Therefore, this *Wands* factor does not support a finding that the present claims lack enablement.

h) the breadth of the claims

The Examiner states:

> h) The breadth of the claims includes thousands of compounds of the instant formula (I) as well as presently unknown compounds embraced by the terms solvates. ... Undue experimentation will be required to practice Applicant's claimed invention.

Office Action, page 10.

Applicant disagrees with Examiner's statement that "....thousands of compounds of the instant formula(I) ...(are) embraced by the term solvate" as lacking enablement commensurate with the claims. As discussed above, the Examiner has presented no evidence that would cast doubt on the truthfulness of statements made by applicant in the current application (*Marzocchi*, *supra*). The solvates of the presently claimed invention are prepared by routine methods well known to those skilled in the arts. The routine nature of these methods is stated by *Caira*, *supra*.

As noted above, even if the amount of experimentation to practice the full scope of the claimed invention might be extensive that does not mean that the amount of experimentation is undue. See *Johns Hopkins Univ. v. Cellpro, Inc,* 47 USPQ2d 1705, 1719 (Fed. Cir 1998) ("test [for undue experimentation] is not merely quantitative...if it is merely routine").

Thus, this *Wands* factor does not support a finding that the present claims lack enablement.

In view of the *Wands* factors discussed herein above, Applicant submits that the weight of evidence clearly supports a finding that claims 1-4, 7-12 and 16-25 comply with the enablement requirement of 35 USC 112, first paragraph. Applicant respectfully requests withdrawal of this rejection.

2. Claim 32, 33, 38 and 40 are rejected under 35 USC 112, first paragraph, as allegedly not being enabled.

The Examiner asserts that the specification does not reasonably provide enablement for the treatment of type I and type II diabetes, obesity and psoriasis.

Claims 32, 33, 38 and 40 have been cancelled rendering the rejection moot. Therefore, Applicant offers no further remarks on this rejection.

CONCLUSION

For the reasons stated above, Applicant asserts that the application is in condition for allowance and that action is earnestly solicited.

Respectfully submitted,

Date: November 5, 2008

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Paper No. 28

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Ex parte JOACHIM GANTE,
HORST JURASZYK,
PETER RADDATZ,
HANNS WURZIGER,
SABINE BERNOTAT-DANIELOWSKI, and
GUIDO MELZER

Appeal No. 2000-0600
Application No. 08/642,268

ON BRIEF[1]

Before WILLIAM F. SMITH, ADAMS, and MILLS, Administrative Patent Judges.

ADAMS, Administrative Patent Judge.

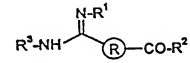
DECISION ON APPEAL

This is a decision on the appeal under 35 U.S.C. § 134 from the examiner's final rejection of claims 1, 2, 5-11, and 13-19. The examiner has indicated that claim 3, the only other pending claim, is allowed. See Final Office Action, page 3.

[1] We recognize appellants' request for an oral hearing (Paper No. 23, received June 23, 1999). However, in accordance with 37 CFR §1.194(c), the Board decided that an oral hearing was not necessary in this appeal.

Claim 1 is illustrative of the subject matter on appeal and is reproduced below:

1. A compound of the formula I

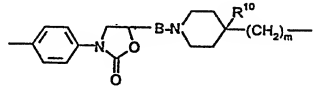


I,

in which

R is

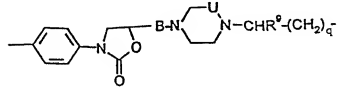
(a)



with B = CH_2, CO or CS, R^{10} = OH or H and

m = 0, 1, 2, 3 or 4; or

(b)



with B = CH_2, CO or CS, U = CH_2 or CO and

R^9 = H, CO_2H or CO_2A, and q = 0, 1, 2 or 3

R^1 is H, A, Ar-CO, A-CO, OH, OA or AO-CO;

R^2 is OH, OA, OAr, OHet, NHOH, NH_2, NHA or NA_2;

R^3 is A-CO, Ar-CO, Het-CO, Het-O-CO, Ar-O-CO, A-O-CO, Ar-SO_2 or A-SO_2;

A is alkyl with 1 to 6 C atoms;

Ar is aryl of 6 to 10 C atoms, or diphenylmethyl or benzyl which are unsubstituted or substituted once, twice or three times by A, F, Cl, Br, I, OA, -O-CH_2-O-, COOA, COOH, CF_3, OH, NO_2, CN, NH_2, O-CO-A, NHA or NA_2; and

Het is a mono- or binuclear saturated, unsaturated or aromatic heterocycle with 1 to 4 N, O and/or S atoms, which can be unsubstituted or substituted once by F, Cl, Br, CF_3, A, OH, OA, CN or NO_2,

and their physiologically acceptable salts and solvates.

The examiner does not rely on prior art.

GROUNDS OF REJECTION

Claims 1, 2, 5-11, and 13-19 stand rejected under 35 U.S.C. § 112, first paragraph, as based on a specification that fails to enable the subject matter of the claimed invention.

Claims 7-11 and 18-19 stand rejected under 35 U.S.C. § 112, first paragraph, as based on a specification that fails to enable the use of the subject matter of the claimed invention.

Claims 1, 2, 5-11, and 13-19 stand rejected under 35 U.S.C. § 112, second paragraph, as indefinite for failing to particularly point out and distinctly claim the subject matter of the claimed invention.

Claims 7-11 stand rejected under 35 U.S.C. § 112, second paragraph, as indefinite for failing to particularly point out and distinctly claim the subject matter of the claimed invention.

We reverse.

DISCUSSION

THE REJECTIONS UNDER 35 U.S.C. § 112, FIRST PARAGRAPH:

Claims 1, 2, 5-11, and 13-19:

The examiner finds (Answer, page 5) appellants "provide no reasonable assurance that [the] piperazion,[ ]piperidino species having 'het' at R2 and/or R3 will all share the requisite profile of activity needed to be operative for practicing the invention." According to the examiner (id.) while certain in vitro tests are disclosed on pages 4-5 of the specification it has "not been shown if a variety of hetero rings at R2,[ ]R3 have been tested or just one or two of the working examples directed to a much narrower scope- ie.[ ]piperidino,[ ]pyridyl,[ ]thienyl and furyl in the R3 group."

We note that the examiner provides no evidence to support her position, instead, the examiner simply concludes that the specification is not sufficient to support the claimed invention. With reference to In re Marzocchi, 439 F.2d 220, 224, 169 USPQ 367, 370 (CCPA 1971), appellants argue (Brief, page 6) "that the burden of proof on this issue has been prematurely shifted to [a]ppellants." We agree with appellants.

Whether the disclosure is enabling, is a legal conclusion based on several underlying factual inquiries. See In re Wands, 858 F.2d 731, 735, 736-37, 8 USPQ2d 1400, 1402, 1404 (Fed. Cir. 1988). As set forth in Wands, the factors to be considered in determining whether a claimed invention is enabled throughout its scope without undue experimentation include the quantity of experimentation necessary, the amount of direction or guidance presented, the presence or absence of working examples, the nature of the invention, the state of the prior art, the relative

skill of those in the art, the predictability or unpredictability of the art, and the breadth of the claims.

We find no analysis of the Wands factors by the examiner. Instead, we find only the examiner's unsupported conclusions as to why the specification does not enable the claimed invention. We remind the examiner that nothing more than objective enablement is required, and therefore it is irrelevant whether this teaching is provided through broad terminology or illustrative examples. Marzocchi, 439 F.2d at 223, 169 USPQ at 369. In the absence of a fact-based statement of a rejection based upon the relevant legal standards, the examiner has not sustained her initial burden of establishing a prima facie case of non-enablement. As set forth by appellants' (Brief, page 7):

> A disclosure which contains representative examples which provide reasonable assurance to one of ordinary skill in the art that the compounds falling within the scope of the claim can be made and possess utility is all that is required, absent any reasons given as to why the statements made in the specification are not accurate.

We agree. The burden of proof does not shift to appellant until the examiner first meets her burden. Marzocchi, 439 F.2d at 223-224, 169 USPQ at 369-370. On this record, the examiner has not met her burden.

Accordingly, we reverse the examiner's rejection of claims 1, 2, 5-11, and 13-19 under 35 U.S.C. § 112, first paragraph.

Claims 7-11 and 18-19:

According to the examiner (Answer, page 5) the "[s]pecification does not adequately teach one 'how to use' the instant compounds for all disorders apparently embraced by the method claims." The examiner finds (Answer, page 5)

that the specification makes reference to a number of disorders, including, "(but ... not limited to) thromboses,[ ]osteolytic disorders, kidney failure [and] antitumor agents." However, the examiner finds (Answer, pages 5-6) that the "nature of testing relied on in the specification does not appear to be art-recognized for treating all such disorders." In this regard, the examiner makes reference (Answer, page 6) to two prior art references – Muller and Smith. However, as set forth on page 3 of the Answer, "[n]o prior art is relied upon by the examiner in the rejection of the claims under appeal." As set forth in In re Hoch, 428 F.2d 1341, 1342 n.3, 166 USPQ 406, 407 n.3 (CCPA 1970) ("[w]here a reference is relied on to support a rejection, whether or not in a 'minor capacity,' there would appear to be no excuse for not positively including the reference in the statement of the rejection"). Accordingly, the examiner's reliance on Muller and Smith is in error.

Notwithstanding the examiner's error, appellants responded to the examiner's argument (Reply Brief, page 4), therefore, we will consider the references to the extent that the examiner and appellants have relied on them. For emphasis, the following quote reproduces in full the examiner's position relative to the cited references (Answer, page 6), "[a]t best,[ ]Muller, provided in an earlier action, suggests a correlation exists for the treatment of thrombosis-see second [to the] last paragraph on p.[ ]113. Note also Smith cited in the specification,[ ]p.[ ]4, for an example of a ligand binding assay that can be used does not make such assertions. See page 12270."

While we find the examiner's point less than clear, we find appellants' response compelling. According to appellants' (Reply Brief, page 4):

> The [e]xaminer's Answer alleges that the assays disclosed in the specification and the examples in the art of record (e.g., Muller) are only art-recognized for, at best, the treatment of platelet aggregation and thrombosis (as is recited, e.g., in cla[i]m 8). However, the [e]xaminer has provided no reasons to cast doubt on appellants' assertion that these models are, in fact, suitable for determining how to treat the diseases encompassed by the claims (e.g., thromboses, osteolytic disorders, kidney failure, tumors). As was discussed in the Appeal Brief, the burden lies with the [e]xaminer to provide evidence which raises doubt about what applicants have disclosed as to the utility of the invention (In re Marzochi et al[.]), and in the absence of such evidence, the rejection cannot be maintained.

Here again we find no analysis of the Wands factors by the examiner. Instead, we find only the examiner's unsupported conclusions as to why the specification does not enable the claimed invention. As set forth above, in the absence of a fact-based statement of a rejection based upon the relevant legal standards, the examiner has not sustained her initial burden of establishing a prima facie case of non-enablement. We recognize appellants' argument (Brief, page 7):

> Again, the [e]xaminer appears to set forth an initial burden upon [a]ppellants to provide tests as to the applicability of the compounds to many of the several embodiments of the claims. Appellants urge that such burden is unfounded in the absence of reasons for doubting the objective truth of the statements in the specification on how to conduct the methods, explanations why the truth or accuracy of such statements are doubted and acceptable evidence or reasoning to back up the assertions that such statements are insufficient."

We again remind the examiner that the burden of proof does not shift to appellant until the examiner first meets her burden. Marzocchi, 439 F.2d at 223-224, 169 USPQ at 369-370. In our opinion, the examiner has not met her burden on this record.

Having determined that the examiner has not met her burden of established a prima facie case of non-enablement, we find it unnecessary to discuss the Melzer Declaration, relied on by appellants to rebut any such prima facie case.

Accordingly, we reverse the rejection of claims 7-11 and 18-19 under 35 U.S.C. § 112, first paragraph.

THE REJECTIONS UNDER 35 U.S.C. § 112, SECOND PARAGRAPH:

The legal standard for indefiniteness under 35 U.S.C § 112, second paragraph, is whether a claim reasonable apprises those of skill in the art of its scope. See, Amgen Inc. v. Chugai Pharmaceutical Co., Ltd. 927 F.2d 1200, 1217, 18 USPQ2d 1016, 1030 (Fed. Cir. 1991).

Claims 1, 2, 5-11, and 13-19:

According to the examiner (Answer, page 3) the "[s]cope of 'solvates' as recited in ... [the] claims is unknown." The examiner finds (id.) "[g]enerally not all solvents can form solvates with all compounds ... [and] it is not routine for any and every type of solvent for form solvate(s) with specific compounds." According to the examiner (Answer, page 4) "[i]n the absence of any guidance in the specification ... or in any relevant prior art,[ ]one cannot readily determine what is and what is not within the instant scope of solvates."

In response, appellants argue (Brief, page 3) "[t]hat all solvents cannot form solvates with all compounds is not seen to be relevant herein. The relevant inquiry is whether it would be known to one of ordinary skill in the art what solvents form physiologically acceptable solvates with the compounds of formula I." In this regard, appellants argue (Brief, page 4), with reference to Hybritech Inc. v. Monoclonal

Antibodies, Inc., 802 F.2d 1367, 1385, 231 USPQ 81, 94 (Fed. Cir. 1986), "the selection of useful solvents and formation of solvates is highly routine in this art. Appellants need not provide in their specification a description of matter which is common and routine in the art. A 'patent need not teach, and preferably omits, what is well known in the art.'" According to appellants' (Reply Brief, page 2) "[b]ecause selection of the appropriate solvents for forming solvates was routine to one of ordinary skill in the art, the metes and bounds of the term were reasonably determinable using only ordinary skill in the art." We agree.

Accordingly, we reverse the rejection of claims 1, 2, 5-11, and 13-19 under 35 U.S.C. § 112, second paragraph.

Claims 7-11:

According to the examiner (Answer, page 4) the "[s]cope of method claims 7-11 is unknown as no particular disorder is recited in these claims only a mechanism of action, namely diseases 'associated with undesirable integrin binding'." The examiner finds (id.) the "claim language is of indeterminate scope since it may read on diseases that are affected by integrin binding ... in ways not yet understood."

In response, appellants argue (Reply Brief, page 3) "[w]hile it is possible that there may be disease conditions caused by undesirable integrin binding which cannot be treated successfully by the compounds of the invention, the existence of a few inoperable embodiments does not render the invention non-enabled or of indeterminate scope." We agree. Cf. Atlas Powder Co. v. E.I. DuPont De Nemours & Co., 750 F.2d 1569, 1576-77, 224 USPQ 409, 414 (Fed. Cir. 1984):

> Even if some of the claimed combinations were inoperative, the claims are not necessarily invalid. "It is not a function of the claims to specifically exclude ... possible inoperative substances...." In re Dinh-Nguyen, 492 F.2d 856, 859-59, 181 USPQ 46, 48 (CCPA 1974)(emphasis omitted). Accord, In re Geerdes, 491 F.2d 1260, 1265, 180 USPQ 789, 793 (CCPA 1974); In re Anderson, 471 F.2d 1237, 1242, 176 USPQ 331, 334-35 (CCPA 1971). Of course, if the number of inoperative combinations becomes significant, and in effect forces one of ordinary skill in the art to experiment unduly in order to practice the claimed invention, the claims might indeed be invalid. *See* e.g., In re Cook, 439 F.2d 730, 735, 169 USPQ 298, 302 (CCPA 1971).

On this record, the examiner failed to provide evidence demonstrating that the number of inoperative combinations is significant enough to force one of ordinary skill in the art to experiment unduly in order to practice the claimed invention.

The examiner also finds (Answer, pages 4-5) "[t]he level of undesirable integrin binding is not synonymous with doses of specific drugs needed for administration to treat a specific disease but rather the underlying physiological contributing factor that may lead to one or more diseases that is present in the host and this quantity is never defined." In response appellants argue (Reply Brief, page 3) that "one of skill in the art would known that 'undesired integrin binding' is an amount of binding which results in disease symptoms, e.g., the disease recited in the specification, so the term is not indefinite." We agree.

Furthermore, appellants argue (Brief, page 4) that "the 'effective' amount of an agent required to inhibit an integrin is the amount of the agent which, in fact, does inhibit the interaction of an integrin with its receptor and/or ligand. Since the amount which provides such activity can be readily determine by one of ordinary skill in the art, the meaning of 'effective amount' is known." Again, we agree with appellants.

On reflection, we find that the examiner failed to meet her burden of establishing that the claims are indefinite. Accordingly, we reverse the examiner's rejection of claims 7-11 under 35 U.S.C. § 112, second paragraph.

REVERSED

William F. Smith Administrative Patent Judge	)
Donald E. Adams Administrative Patent Judge	) BOARD OF PATENT) APPEALS AND) INTERFERENCES
Demetra J. Mills Administrative Patent Judge	)

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Crystalline Polymorphism of Organic Compounds

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Crystal polymorphism is encountered in all areas of research involving solid substances. Its occurrence introduces complications during manufacturing processes and adds another dimension to the complexity of designing materials with specific properties. Research on polymorphism is fraught with unique difficulties due to the subtlety of polymorphic transformations and the inadvertent formation of pseudopolymorphs. In this report, a summary of thermodynamic, kinetic and structural considerations of polymorphism is presented. A wide variety of techniques appropriate to the study of organic crystalline polymorphism and pseudopolymorphism is then surveyed, ranging from simple crystal density measurement to observation of polymorphic transformations using variable-temperature synchrotron X-ray diffraction methods. Application of newer methodology described in this report is yielding fresh insights into the nature of the crystallization process, holding promise for a deeper understanding of the phenomenon of polymorphism and its practical control.

Keywords: Crystal polymorphism, Pseudopolymorphism, Crystallization.

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1 Introduction

The protean nature of a chemical substance, reflected in its ability to crystallize in different structural arrangements (polymorphs), has since its discovery [1] been a source of both fascination and frustration for chemists. At a given temperature and pressure, only one polymorphic form of a substance is thermodynamically stable, all other forms being metastable. Since the rate of transformation of metastable polymorphs to the stable one may be slow, it is quite common to encounter several polymorphs of a single compound under normal laboratory conditions. Organic compounds tend to form different polymorphs owing to weak, non-directional intermolecular interactions which exist in the solid state. When a compound can be isolated in different polymorphic modifications, each with the potential of possessing unique properties (solubility, density, melting point, enthalpy of fusion, chemical reactivity, electrical conductivity, to name a few), the chemist, pharmaceutical chemist, or chemical engineer is presented with a degree of flexibility of choice for a particular application. Balanced against this flexibility, however, are the considerable practical difficulties that can arise, both in ensuring reproducible preparation of a specific polymorph and, during the lifetime of its application, preventing its spontaneous transformation to an undesirable form. Since free energy differences between polymorphic forms of a given substance are generally around a few kJ mol^{-1} [2] and the process of crystallization is affected by many physical parameters (e.g. nature of the solvent, cooling and stirring rates, temperature, pressure, presence of impurities), minor variations in preparative conditions can tip the balance in favour of crystallization of a polymorph which is not necessarily the thermodynamically stable one. This element of unpredictability in the outcome of the crystallization process has serious implications for solids design in crystal engineering [3], where the required specificity of molecular organization in the crystalline state is crucial.

Various aspects of organic crystalline polymorphism and its occurrence in the fine chemicals, pharmaceuticals and other industries have been the subjects of several recent reviews. With varying degrees of overlap, these reviews can be roughly grouped into the following, according to their focus: thermodynamic and kinetic aspects [4–7], structural aspects [2, 3, 8–17], methodology [18–27], the crystallization process [28–34] and polymorphic control [35, 36]. The reader is referred to the above for a comprehensive view of what is a rather pervasive phenomenon in chemistry and whose pursuit is currently enjoying an upsurge of interest from solid-state researchers [5].

This report describes some recent developments in the understanding of the thermodynamic, kinetic and structural aspects of organic crystal polymorphism with an emphasis on the application of newer methodology used for its study, since this is one of the areas in which significant progress has been made in recent years. Numerous examples of polymorphic systems are described to illustrate the applications of both older and newer techniques for their investigation. These include studies of pseudopolymorphism manifested by hydrates and solvates of the parent organic molecule. Finally, the crucial question of

control in polymorphism is briefly addressed with a view to illustrating current strategies and their implications for the design of solids.

2 Crystal Polymorphism – Theoretical Principles and Practical Implications

2.1 Background – The Role of Polymorphism in the Production of Materials

Many of the inconsistencies encountered in product performance in the chemical, chemical engineering, pharmaceutical, food and related industries can be attributed to polymorphism. An important example is inconsistent behaviour of drug substances upon dissolution which may have a direct influence on bioavailability. This arises because different polymorphic forms of the same drug may have solubilities which differ by an order of magnitude [24]. Inadvertent production of the 'wrong' polymorph at the crystallization stage following synthesis or at any of the intermediate processing stages can therefore result in pharmaceutical dosage forms which are either ineffective or toxic [37, 38]. Spontaneous polymorphic transformations mediated by solvents is common [4] and liquid preparations of metastable drugs frequently lose their effectiveness due to precipitation of less soluble, thermodynamically more stable polymorphs or pseudopolymorphs. A case in point is the antiprotozoal agent metronidazole benzoate which, when stored as an aqueous suspension below 38 °C is metastable, leading to precipitation and growth of the insoluble monohydrate [39, 40].

Dunitz and Bernstein [5] have recently documented several cases of "vanishing" polymorphs. These are usually metastable forms which, despite their thermodynamic instability, may have crystallized preferentially due to more rapid nucleation. Such metastable forms may persist and be used for many years before being "displaced", when a thermodynamically more stable form is prepared. Attempts to regenerate the original polymorph are frequently met with failure. Specific compounds with such a history include e.g. 1,2,3,5-tetra-*O*-acetyl-*β*-D-ribofuranose, benzocaine picrate and xylitol. This disturbing phenomenon extends to pseudopolymorphs. A previously known monohydrate of the antibiotic ampicillin has not been obtained since the appearance of the trihydrate [24]. A possible explanation for this behaviour is that after minute particles of the stable polymorph enter the environment, they eventually become widely disseminated ("planetary seeding" [5]) and serve as nuclei promoting crystallization of their own kind exclusively.

Manufacturing processes including crystallization scale-up, drying, heating, compression and milling can induce polymorphic transformations [24] and it follows that careful quality control is necessary at all stages to monitor undesirable changes. Systematic investigation of a compound to determine whether it is prone to polymorphism, as well as the nature of the polymorphism (enantiotropic or monotropic) [23], is routine practice in pharmaceutical pre-formulation studies. Identification of the different polymorphic forms of a drug substance, determination of their chemical and physical properties, thermodynamic

stabilities, and temperatures and rates of interconversion are essential for ensuring drug preparations with reproducible behaviour [24]. Already, legislation requiring drug manufacturers to provide information relating to the occurrence (or apparent absence) of polymorphism in their products has been introduced [41]. Demonstrating the absence of a tendency to polymorphism is not easy; most substances when investigated for a sufficiently long time will reveal more than one polymorph [42].

Successful preparation of crystals of organic compounds having special properties (e.g. second-harmonic generation, metallic conductivity) may hinge on polymorphism, only one polymorph of the compound in question displaying the desired property. Bernstein has recently described representative systems which clearly illustrate the relationship between a polymorphic structure (a crystal architecture characterised by well-defined molecular interactions) and the unique physical properties which that structure confers on the solid material [13].

These remarks serve to emphasise some of the more important practical implications and consequences of polymorphism. Overcoming the problems encountered requires a deeper understanding of the processes of nucleation, crystal growth and polymorphic transformation. Several recent studies relating to these topics are reviewed in the next section.

2.2
Crystallization and Polymorphic Transformations – Thermodynamic and Kinetic Considerations

Crystallization of a specific polymorph from a melt, solution or vapour, commences with nucleation, i.e. the formation of a critical "embryonic" nucleus which is the structural blueprint for subsequent development and growth of the macroscopic crystal. The factors determining nucleation rate (e.g. the associated Gibbs free energy of activation, molecular volume, interfacial energy) generally differ for polymorphs of the same substance [4]. Since, in a supersaturated solution, nuclei of all possible polymorphs of the dissolved substance may be imagined to exist [36], the outcome of crystallization is kinetically complicated by competitive nucleation processes. Thermodynamic considerations of polymorphic crystallization include Ostwald's law of stages [4, 43], according to which, at high supersaturation, the first form which crystallizes is the thermodynamically least stable (most soluble) form. This form subsequently dissolves and transforms into a more stable one. The cycle continues until only the thermodynamically stable (least soluble) polymorph remains. The practical implication is that it should be possible to isolate the different polymorphs of a given compound at different levels of solution supersaturation and hence exercise some control over the crystallization process.

As regards polymorphic transformations in general, two types are distinguished, namely enantiotropic and monotropic [23]. These can be described in terms of the Gibbs free energy G, which has a minimum value for the thermodynamically stable phase of a polymorphic system and larger values for metastable phases and is such that the polymorph with the higher entropy will tend

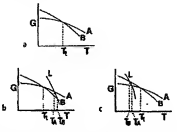


Fig. 1a–c. Gibbs free energy vs temperature for: a a dimorphic system, exhibiting: b enantiotropy; c monotropy

to become the stable form at higher temperature (species B in Fig. 1 a). Above T_t (the transition temperature), B is the stable polymorph while A is metastable and vice versa at temperatures below T_t. In an enantiotropic system (Fig. 1b), the free energy curve for the common liquid phase L intersects the A and B curves at $T > T_t$. In this case, the lower melting form (A) is stable at $T < T_t$, the higher melting form is stable at $T > T_t$, and the transition between the two forms is in principle reversible. Since transition temperatures in practice are often in the range 20–200°C, one practical implication of enantiotropy is that conversion of one polymorph into another may be favoured during routine manufacturing processes [24]. On the other hand, for a system displaying monotropy (Fig. 1 c), curve L intersects those for A and B below T_t and the higher melting form (A) is always the thermodynamically stable one. Thus, below the melting point, only one form is stable and the other metastable. In practice, if a desired metastable polymorph is obtained during manufacture, it can revert to the stable polymorph under suitable conditions (e.g. in suspension, via solvent-mediation, or during compression). It follows that to prepare a specific polymorph and be aware of its possible fate during handling, it is advantageous to know the transition temperatures and thermodynamic stabilities of all the forms that may appear in the system [24].

The general considerations above highlight the importance of nucleation and the role of environmental conditions (e.g. solvent, temperature) in the crystallization of polymorphs as well as their interconversions. These areas continue to be the subject of intense interest especially in the context of polymorphic control in crystallization.

Some fundamental aspects of the nucleation process have been investigated by molecular dynamics (MD) methods. In a recent review [44] the advantages and limitations of molecular cluster models in simulating the dynamics of nucleation and phase changes have been discussed. In this approach, molecular dynamic simulations are correlated with experimental nucleation rates extracted from electron diffraction patterns of molecular supersonic jets. The dynamics of freezing of ammonia, CCl_4 and water, and the phase transformations of *t*-butyl chloride have been analysed. A useful feature of the MD computational

approach is visual representation of phase transformations. Figure 2 illustrates MD-derived images of a crystalline cluster of 188 molecules of *t*-butyl chloride at various stages of freezing. MD simulations show that when sufficiently supercooled, the tetragonal phase spontaneously transforms to a lower temperature, ordered monoclinic phase. Diffraction patterns computed from the MD molecular packing were consistent with experimental neutron powder patterns for this phase.

Other investigators [45] have recently designed a numerical model to describe nucleation and growth of polymorphs with the aim of calculating the temporal sequences of precipitation and phase transformation in metastable solutions of polymorphic substances. Another group has recently modelled the formation and aggregation of polymorphs in continuous precipitation [46]. Consideration was given to the simultaneous growth and agglomeration of two different polymorphs as well as the case of nucleation of a single polymorph which subsequently transforms into a second one. The results indicated that the ratio of the nucleation rates, the ratio of the growth rates, and the aggregation tendencies determined polymorphic product composition as well as particle size distributions. This study is important since simultaneous precipitation of different polymorphs is encountered frequently in industrial crystallizations.

A mathematical phase-field model for the kinetics of isothermal polymorphic crystallization has recently been proposed [47], according to which crystallization involves rapid relaxation of the metastable state followed by nucleation and growth of the polycrystalline phase. Computer simulations were used to obtain results which could be tested experimentally using X-ray scattering experiments. Growth rates of different polymorphic polymers have also been investigated [48]. Simultaneous development of spherulites of different polymorphs occurs at different rates under isothermal conditions. From observation of interspherulitic boundaries between the α- and γ-forms of polypivalolactone,

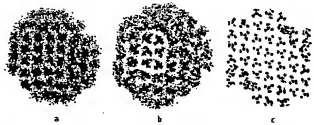


Fig. 2a–c. Images of a crystalline cluster of *t*-butyl chloride molecules at various stages of cooling, looking down the threefold molecular axis: a orientationally disordered tetragonal phase at 130 K; b nucleus of monodinic phase growing in tetragonal phase at 80 K; c ordered monoclinic phase at 50 K after transformation. Surface molecules tend to be disordered at all temperatures. (Reprinted with permission from [44], copyright 1995 American Chemical Society)

their relative growth rates could be determined. The thermodynamics and kinetics of crystallization of large molecules from solution have been discussed [49]. Modelling of the crystallization of protein molecules indicated that the concepts and numerics of colloid stability theory are appropriate.

Recent studies of polymorphic transformations in organic crystals mediated by melt, solution and interface have been reviewed [4]. Interface-mediated transformation has only recently been recognized as a distinct mode of polymorphic transformation. It involves nucleation and growth of a new polymorph through mass transfer across the interface connecting single crystals of two different polymorphs and it differs from solid-solid transformations in that a microscopic solution layer is required as an interface. Transformations mediated by melt, solution and interface are usually more rapid than solid-solid transitions; for the latter, the activation energy is larger due to the fact that nucleation and growth of the new phase within a second phase involve diffusion and structural rearrangement at the reaction interface. The fundamental thermodynamic relationships governing polymorphic solid state transitions have been reviewed [6]. It has also been pointed out that the mechanisms of polymorphic transitions in molecular crystals are largely unknown, though order-disorder transitions are understood in reasonable detail [5]. An important technique for studying solid-solid transformations is thermal analysis and a review of the basic thermodynamic principles for interpreting thermal analysis data for both polymorphic and pseudopolymorphic systems has appeared [23]. Kinetic and thermodynamic aspects of the thermal decomposition of inclusion compounds (a special class of pseudopolymorphs) have also recently been discussed [50].

Theoretical and experimental studies of the role of solvent on polymorphic crystallization and phase transformations abound in the literature of the last few years and some pertinent examples are described here. For solvent-mediated transformations, the driving force is the difference in solubility between different polymorphs. An important earlier paper on the kinetics of such phase transformations [51] described a model featuring two kinetic processes in solid to solid phase changes via a solution phase, namely dissolution of the metastable phase and growth of the stable one.

The effect of solvent on the crystallization of polymorphs has recently been investigated [43] using as a model compound the antibacterial sulphathiazole whose four known polymorphs are well characterised. The study, whose express intention was to test the Ostwald law, involved crystallization of the pure polymorphic forms of the drug, solubility measurements and crystallizations from various solvent systems. Systematic variation of supersaturation was employed in an attempt to crystallize each of the four forms of sulphathiazole, as predicted by the Ostwald law. Solubility studies showed Form I to be the most soluble form, followed in order by Forms II, IV and III. The supersaturation crystallizations using acetone, acetone-$CHCl_3$ (3:2), *n*-propanol and water, revealed that only the acetone-$CHCl_3$ system yielded results in accord with theory, Forms I, III and IV being isolated from it by varying the supersaturation. Crystallization from *n*-propanol, for example, yielded only Form I at all supersaturation levels. Thus, the finding that some solvents selectively favour the crystallization of a

particular form (or forms) indicated that, while supersaturation is an important factor determining crystallization of polymorphs, the solvent may play a dominating role which is not thermodynamic in nature, but rather kinetic. The proposed mechanism, namely selective adsorption of different solvent molecules on specific faces of particular polymorphs, is consistent with that suggested earlier [52] for the effects of additives and solvents on crystal morphology. Such specific adsorption might result in inhibition of nucleation of certain polymorphs or retardation of their growth, allowing other, thermodynamically less favoured, polymorphs to crystallize instead.

The mechanism of a solvent-mediated transformation may change with complete change of solvent, or more subtly, when a gradual change in polarity is effected by dilution of the original solvent with another. The effects of the solvent systems water [53] and ethanol/water [54] on the crystallization of L-histidine polymorphs have been investigated. In aqueous solution at the isoelectric point, it was found that both the A and B polymorphs precipitate with a nearly constant ratio over a wide concentration range. However, slow transformation from B to A (which does not occur in the absence of solvent) was observed and pure A could eventually be isolated. This transformation involves smooth growth of the stable A polymorph and dissolution of the metastable form B. The ratio of the rate constants for the appearance of A and the dissolution of B indicated a growth-controlled mechanism for the transformation. In subsequent experiments investigating the effect of added ethanol, however, it was found that the fraction of polymorph A in precipitates decreased rapidly with increasing volume fraction of ethanol in the mixture, and pure B could be obtained when this fraction was 0.4. An explanation for the change in growth mechanism with added ethanol, based on the decreased concentration of polymorph A_s, was proposed [54].

This type of behaviour is not confined to polymorphs but may extend to pseudopolymorphic forms such as hydrates and solvates. A recent case of solvent-mediated phase transformation involved polymorphic and pseudopolymorphic forms of thiazole carboxylic acid [55], where the transformation is again sensitive to the composition of the mixed solvent. Three forms of the compound are known, an anhydrous form, a 0.5 hydrate, and a 1.5 hydrate. In 50–80% solutions (% = vol.% MeOH-H_2O), transformation of the 1.5 hydrate to the 0.5 hydrate was observed while transformation to the anhydrous form occurred in 85–100% solutions. No transformation occurred in 0–30% solutions. Detailed study of a solvent-mediated polymorphic transformation has also been carried out for the antiulcerative agent cimetidine [56] for which seven polymorphic forms are known. An important feature of this study was the systematic use of seed crystals to induce crystallization at different supersaturation ratios.

The possibility of relating solvent effects to polymorphic crystallization at the molecular level may be realized when the individual crystal structures of the polymorphs are known. An analysis of this kind was carried out for the anti-inflammatory drug piroxicam [57] which was found to crystallize as the α-polymorph from proton donor and basic solvents, but as the β-polymorph from non-polar solvents. On the assumption that crystallization of the drug requires

release of solvent molecules from acceptor and donor sites on the molecule, it was possible to reconcile the observed intermolecular hydrogen bonding arrangements in the crystals (infinite chains in α-, cyclic dimers in β-) with probable sites of solvation on the piroxicam molecule in solvents of different polarities. In this way, formation of the α- and β-polymorphs from different solvent systems could be rationalised. Combination of polymorphic crystal structural data with appropriately detailed solvation models could be a useful adjunct to the existing methods of rationalising or predicting the outcome of polymorphic crystallization from solution.

2.3 Polymorphism – Structural Considerations

Dunitz has recently developed the theme of the crystal as an ordered supramolecular entity [2]. From this perspective, different polymorphic modifications of a given compound may thus be regarded as "supramolecular isomers". In discussing the possible structural arrangements occurring in crystals of polymorphs, a convenient distinction may be made [3] between rigid molecules (e.g. planar chloro-aromatics) and those with conformational flexibility (e.g. *N*-benzylideneanilines). In the former case, different polymorphic structures frequently display common features such as similar intermolecular directional contacts, layer stacking and unit cell dimensions which are related in a simple way. Conformational polymorphism, the existence of different conformers of a flexible molecule in the various crystal structures [58], may be expected to occur when the conformational energy minima differ by less than about 8 kJ mol^{-1}. If the energy barriers separating these minima are sufficiently low, these conformers may co-exist in solution and slight variations in crystallization conditions may lead to their individual isolation as conformational polymorphs [11]. Systems of this type have been exploited to study both the influences of the crystalline environment on molecular conformation as well as the properties of molecules which depend strongly on conformation [13]. An example of conformational polymorphism in which the structural arrangements are dictated by non-directional van der Waals forces only is shown in Fig. 3. The molecule in question is probucol, a drug used to control blood-cholesterol levels. Here, intermolecular hydrogen bonding between hydroxyl groups in the crystals is prevented owing to intramolecular steric crowding of these groups by neighbouring *t*-butyl substituents. The molecules adopt distinctly different conformations in the two polymorphs [59], the more symmetrical conformation approaching point symmetry C_{2v}.

Figure 4 shows representative hydrogen bonded (N-H···O, C-H···O) layers of planar nitrofurantoin molecules occurring in the α- and β-polymorphs [60] which are triclinic and monoclinic, respectively. In this system, the molecular conformations in the two polymorphs are indistinguishable but the symmetries of their intermolecular hydrogen bonding schemes differ significantly. The common molecular conformation shown here occurs in five modifications (two polymorphs and three pseudopolymorphs) of this compound [61]. The three-dimensional crystal structures of the polymorphs result from close stacking

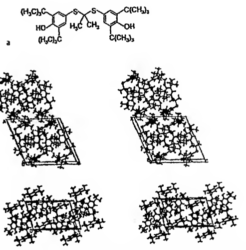


Fig. 3. a Molecular structure of probucol; b stereoview of the crystal structure of Form I; c stereoview of the crystal structure of Form II. (Adapted from [59] with permission)

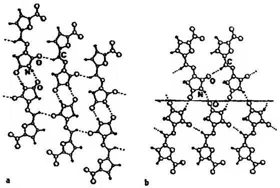


Fig. 4a,b. Hydrogen bonded layers in nitrofurantoin polymorphs: a α-form; b β-form (Adapted from [60] with permission)

(~ 3.2 Å) of the respective layers shown in Fig. 4. This example conveys an idea of the variations in polymorphic structural arrangements that are possible with molecules containing hydrogen bonding functionalities.

During the last few years, the development of graph set analysis [16] has greatly facilitated the visualisation and comparison of polymorphic structures. Here, hydrogen bonding networks are classified as belonging to one of four distinct patterns, each specified by a designator (G in general): intramolecular (S), chains (C), rings (R), or other finite patterns (D). Further specification of the number of donor (d) and acceptor (a) atoms as well as the total number of atoms (n) comprising the pattern yields a concise and informative description of the hydrogen bonding arrangement, $G_d^a(n)$. A simple illustration of the use of graph set descriptors is given in Fig. 5 for a polymorph of thalidomide (space group C2/c, Z=8) [62]. Alternating hydrogen bonded ring motifs exist in this polymorphic structure as a result of bifurcated hydrogen bonding involving the N-H group. In contrast, the other known racemic modification of thalidomide (space group $P2_1/n$, Z=4) [63] contains only centrosymmetric dimers of the type $R_2^2(8)$. Application of graph set analysis to three polymorphs of iminodiacetic acid [64] has led not only to facile comparison of the crystal structures, but has also provided a basis for concise description of the polymorphic transformations occurring in that system. Thus, e.g. transformation of one polymorph of the acid into another is simply described as a conversion of $R_2^2(4)$ into $R_2^2(8)$. It has been pointed out that graph set analysis of hydrogen bonded systems also provides a means for seeking systematic correlations between the resultant patterns that demonstrate "hydrogen bond pattern functionality" [16]. This concept may be useful in the prediction of crystal structures as well as for the design of materials with desired supramolecular features. Extension of the graph set approach to encompass intermolecular atom-atom interactions other than hydrogen bonding for the classification of polymorphic crystal structures is envisaged as a natural and desirable development and more widespread use of this classification can be expected in future.

Elucidation of detailed polymorphic structural features and structural changes accompanying polymorphic transformations relies heavily on the single crystal X-ray diffraction technique. The inability to produce single crystals in

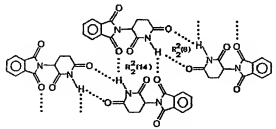


Fig. 5. Graph set notation for hydrogen bonded ring motifs in a polymorph of thalidomide

the laboratory (especially for metastable polymorphs) has hindered progress in research on polymorphism. If, however, a polymorph is available in a powdered crystalline form and a computational method exists for predicting possible three-dimensional crystal structures, then the latter can be used to generate computed X-ray powder patterns for comparison with the experimental pattern. While the scope of the present review does not permit detailed exposition of the approaches and algorithms employed in crystal structure prediction, a summary of some recent developments, especially as they relate to polymorphism, is in order. The studies of polymorphism and crystal structure prediction are indeed two facets of the same topic [65]. Additional powerful motivations for predicting crystal structures include a better understanding of the crystallization process as well as the design of new solid materials. The reader is referred to several recent treatments of crystal structure prediction [66–70]. Comments on some of these methods and their applications follow.

In the atom-atom potential (AAP) method as implemented in the program PROMET [66], symmetry operations (e.g. a screw axis, centre of inversion) appropriate to the chosen space group, are applied to a molecule in fixed conformation (possibly optimised previously by a molecular mechanics calculation) to generate molecular clusters. Following a search for the most stable clusters on the potential hypersurface (calculated using empirical atom-atom potentials), translation is applied to generate one or more periodic structures. Packing energies are computed and acceptable structures are optimised. This is a very useful means of generating a series of polymorphic structures for a given molecule. In this approach, consideration is usually given to only the most populous space groups (93% of organic molecules being confined to 18 space groups [71]) thereby running a small risk of an incorrect choice. If an experimental X-ray powder pattern of the material is available, the correct crystal structure may be identified by comparison with computed powder patterns from the candidate structures generated by PROMET. In a study using this procedure [72] several literature cases were selected, for each of which the crystal structure of one polymorph had been determined, and mention was made of the existence of a second polymorph whose crystals were unsuitable for complete structural elucidation by X-ray analysis. Only unit cell and space group data were available for these undetermined polymorphs. Each starting molecular conformation was assumed to be the same as that in the corresponding fully characterised polymorph and was submitted to the PROMET procedure. In all cases, satisfactory structures were generated, with predicted unit cell data in good agreement with the experimental ones and with packing energies in the expected ranges. These results represent authentic crystal structure prediction, assisted by partial X-ray data. The method is therefore an alternative to direct methods of structural solution and also implies that, provided cell and space group information can be acquired, full structure determination without diffraction data is feasible. The authors of this study are less optimistic as regards true ab initio crystal structure prediction for numerous reasons, among them that packing energies for polymorphs of the same compound are always very similar, rendering the choice of the correctly computed structure difficult in the absence of other data, and that the occurrence of polymorphs containing mole-

cules in different conformations is common. The point was also made that the correct energy ordering of polymorphic structures by computational methods may bear no relation to the experimental situation, where kinetic factors may determine which polymorph actually crystallizes under given conditions.

The AAP approach, with some variations, has recently been applied with varying degrees of success to the prediction of several other structures including, in order of increasing molecular complexity, high pressure solid phases of benzene [73], the three polymorphs of sulfanilamide [74], and the low and high temperature phases of poly(p-hydroxybenzoic acid) [75].

An ab initio molecular packing analysis procedure (program mpa) which avoids any prior assumption of space group symmetry has recently been described [76]. Only the molecular structure and the force field are required as inputs and the program finds intermolecular energy minima for packed arrangements for any given number of molecules comprising the asymmetric unit. Space group symmetry operations are predicted in this procedure and successful application to the crystal structures of urea and benzene were reported. It is significant that the energy minimisation for the urea structure converged to the correct space group ($P\bar{4}2_1m$) which has a frequency of occurrence of only 0.39%.

Quantum mechanical methods have also been applied to crystal structure prediction. A recent example involved the use of ab initio crystal field methods with the SM (supermolecule) model and the PC (point charge) model applied to the three known polymorphs of glycine [77]. Comparison of the optimised structures with published X-ray structures for these forms indicated that the quantum-mechanically based SM model employing a 15-molecule cluster produced results in better agreement with experiment than the PC model which describes the crystal environment purely electrostatically.

Despite the varying degrees of success attainable by such computational methods, a survey of the recent literature on this subject seems to indicate that crystal structure prediction by theoretical methods is more rapid, has greater chances of success, and consumes far fewer computing resources when coupled with other techniques which provide additional experimental data for the crystal in question. (Examples of such combined studies are discussed in Sect. 3.2). At the same time, it can be argued that successful ab initio crystal structure prediction (i.e. assuming only the molecular structure as given) by whatever means possible in the future, would represent a very significant advance in the understanding of the fundamentals of the crystallization process. Regarding the feasibility of crystal structure prediction in general, some philosophical and technical points have been discussed, together with excellent practical recommendations for a programme of experimental and theoretical studies for elucidating the basic principles of organic solid-state chemistry [78]. This is seen as a prerequisite to the solution of the problem of crystal structure prediction.

Some new insights into the nature of organic crystal polymorphism have been gleaned from a recent systematic analysis [79] of data for polymorphic structures retrieved from the Cambridge Structural Database. A total of 345 crystal structures were reduced to 163 clusters (a cluster referring to a group of two or more polymorphs of the same compound). These clusters comprised 147

with 2, 13 with 3, and 3 with 4 partners. Differences in molecular properties (P) (e. g. density, molecular volume, packing coefficient, AAP-calculated packing energies and other thermodynamic properties) were computed between cluster members (i, j) as $\Delta P = P_j - P_i$ or $\Delta P = 100(P_j - P_i)/P_j$ ($j > i$) for a total of 204 data points. Both monovariate and bivariate statistical analyses were performed on the data, yielding several revealing trends and correlations. Histograms of differences in properties between polymorphic pairs, shown in Fig. 6a–c, indicate respectively that differences in crystal packing energies, densities and lattice vibrational entropies for polymorphs are rather small while Fig. 6d reveals that an appreciable proportion (actually 18%) of polymorphs have $Z' > 1$ (where Z' is the number of molecules in the crystal asymmetric unit). Some further important conclusions drawn from this study are as follows: both calculated and experimental values for the relative stability of crystal polymorphs are currently subject to large uncertainties; polymorphs with $Z' > 1$ are as stable, or even more stable than those with $Z' = 1$; higher crystal density is, as expected, found to

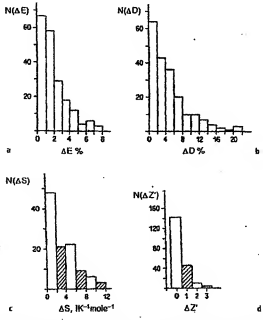


Fig. 6a–d. Histograms of differences in properties between polymorphs: a ΔE (packing energy, %); b ΔD (density, %); c ΔS (lattice-vibrational entropy, J K^{-1} mol^{-1}); d ΔZ' (no. of molecules in the asymmetric unit). (Reprinted with permission from [79], copyright 1995, American Chemical Society)

correlate with higher packing energy. Finally, from the observation that 24 % of the polymorphic pairs analysed comprised both a centrosymmetric and a non-centrosymmetric partner, it was concluded that the link between molecular properties and centrosymmetry of crystals is evidently weak. The reader is referred to this seminal study [79] for the full exposition of the above conclusions and their justification, as well as further perceptive observations on the phenomenon of polymorphism.

3
Methodology for the Study of Crystal Polymorphism

3.1
Review of Preparative Methods

Research on the polymorphism of a new molecular entity normally commences with experimental screening which can indicate the occurrence of more than one crystalline form of the substance. An inexpensive method of such testing is hot stage microscopy (HSM), which has been used very extensively and effectively by a leading proponent [80] for many years to provide preliminary indications of the presence of crystalline polymorphic and pseudopolymorphic (solvated), as well as glassy (amorphous) forms, all of which may have practical utility. Pseudopolymorphic forms are molecular adducts containing solvent of crystallization and have been classified [37] as stoichiometric solvates and non-stoichiometric inclusion compounds possessing channel, layer or cage (clathrate) structures. Procedures for detecting the existence of multiple forms by HSM have been outlined [37, 81]. These include, for example, the observation of solid-solid transformation upon heating the substance, observation of transformation (spontaneous or mechanically induced) following the freezing of a melt, and detection of gas evolution as bubbles from pseudopolymorphs immersed in silicone oil during heating (indicative of a solid → gas + solid transformation). Once the existence of multiple forms is established, practical methods for the preparation of specific forms on a larger scale may be explored. Frequently, recrystallization of the compound from solvents or solvent mixtures spanning a wide polarity range is effective in producing several of the different forms in sufficient quantity for complete characterisation by the analytical methods to be discussed. Most pseudopolymorphs are prepared by crystallization of the parent organic compound from the respective solvent, whereupon the latter becomes incorporated in the new crystal. Recrystallization from a mixed solvent system may yield a pseudopolymorph containing either or both solvents. Exposure of the parent organic compound to vapours may also result in the formation of pseudopolymorphs (as occurs e.g. when anhydrous drugs react with atmospheric water to form hydrates). This is exemplified by the drug indomethacin, for which four polymorphic forms (I–IV) have been identified [82]. Crystallization of the commercially available Form I from over fifty solvents yielded Forms I, II, IV and mixtures of these, as well as a number of pseudopolymorphs [83]. To ensure subsequent reproducibility in the preparation of specific forms by crystallization, careful attention to the details of solvent purity, degree of solu-

tion agitation, temperature, supersaturation and the rate of cooling of the solution is necessary. Detailed methods for isolating metastable polymorphs from the melt or from solution have been reviewed [81].

Desolvation of a pseudopolymorphic form by controlled heating leads to the formation of a polymorph or a mixture of polymorphs of the parent compound, hence providing an additional route to isolation of such species. For example, for indomethacin quoted above, twelve pseudopolymorphs with the general formula indomethacin · $(solvent)_n$ ($n=0.2-1.1$) were isolated [83] and their products of desolvation were characterised. Whereas mixtures of Forms I and II resulted from heating the benzene, CCl_4, $CHCl_3$ and toluene pseudopolymorphs, pure Form II was obtained from desolvation of the acetone pseudopolymorph. The most soluble (and pharmaceutically desirable) polymorph, Form IV, was obtained in a pure state by desolvation of the pseudopolymorph containing methanol.

A technologically very important and potentially beneficial feature of some polymorphs obtained in this way is the possibility that they may acquire altered rheological or other properties (flowability, texture, particle size distribution, compressibility) when compared with the same polymorphic crystalline forms obtained by direct crystallization from solution. Two examples of considerable pharmaceutical relevance may be cited, namely those of lactose and paracetamol. The lactose used as an excipient in pharmaceutical tablets and capsules is α-lactose monohydrate. It was found that thermal dehydration of this species or desiccation of α-lactose containing methanol yielded a stable product with superior binding properties and excellent flowability [84]. Tablets prepared by compaction of the stable product had an overall porosity nearly equal to those of tablets prepared with the original materials. The very poor compression abilities of paracetamol prompted an investigation of solvation/desolvation as a process for preparing pure paracetamol with improved properties [85]. A crystalline hemisolvate of paracetamol was prepared by cooling a hot saturated solution of the drug in dioxane. Desolvation of this species yielded pure paracetamol with significantly improved technological properties including flowability, die filling, and hardness/pressure profile. A crucial point is that this improvement was not attributable to the production of a new polymorphic form of the drug; the X-ray powder diffraction pattern of the desolvated material was the same as that of the commercially available, monoclinic polymorph of paracetamol. However, detailed examination with scanning electron microscopy revealed that desolvation produces material with an unusually porous, sintered-like texture which lends itself to compression more readily than other forms of the drug. Interestingly, solvation/desolvation using other oxygen-donor solvents (e.g. acetone, cyclohexanone) yielded paracetamol in the same (monoclinic) form but failed to produce paracetamol crystals with the required texture. An earlier review on polymorphism [37] lists several drug pseudopolymorphs whose desolvation leads to significant particle-size reduction (or micronization) of the ensuing polymorphic crystallites. This usually results in improved dissolution and tableting properties of the polymorph.

Mechanical grinding and compression of compounds represent another possible route to polymorphs. In the former case, the local pressures induced by

the mechanical stress may initiate the transformation of the original polymorph into another crystalline form. From an industrial viewpoint, grinding and compression are attractive processes, being relatively inexpensive and requiring no solvents. In addition, complete polymorphic conversion may be effected in very short times (several minutes in some cases). Recent examples include the production of polymorphic Form I of sulphathiazole by planetary ball-milling of Form III [86], the transformation of metastable Form I of caffeine into the stable Form II by either grinding or compression [87] and production of Form I of probucol by manual trituration of Form II [59]. The role of grinding in the design of pharmaceutical dosage forms has been investigated and the effects of increased specific surface areas and enhanced solubilities following mechanical action have been noted [88]. The same group reported the effects of environmental temperature and compression energy on the polymorphic transformations of the antidiabetic chlorpropamide [89] and discussed the relation between the polymorphic transformation pathway during grinding and the physicochemical properties of bulk powders of cephalexin, chloramphenicol palmitate and indomethacin [90]. Local temperature variations during grinding may also play a role in effecting polymorphic transformation and it is desirable to separate the two influences. This was recently achieved with cortisone acetate by cryogrinding at 78 K, during which the monoclinic form transformed to an orthorhombic form in ten minutes purely by mechanical effects [91].

Prolonged mechanical grinding of a crystalline compound may produce material in an amorphous (or glassy) state which, due to the lack of long-range internal order of the constituent molecules, displays a broad melting temperature range and a diffuse X-ray diffraction pattern. A discussion of polymorphism without reference to amorphism would represent serious neglect of an important aspect of phase behaviour. The amorphous state represents the thermodynamically least stable form of the compound, which consequently has a tendency to revert to a more stable form. The amorphous material is also the most soluble form of the compound and this property is used to advantage in pharmaceutical preparations in cases where the solubility of the crystalline form of the drug is low, leading to poor systemic absorption. A well known example is the antibacterial novobiocin acid, for which the solubility of the amorphous (and therapeutically active) form is ten times that of the crystalline (inactive) form [92]. However, use of an amorphous form in a suspension may require addition of another component to suppress spontaneous transformation to a thermodynamically more stable form. For novobiocin acid preparations, the additives methylcellulose and polyvinylpyrrolidone are successful in this respect. It seems likely that the amorphous state may attain even greater practical significance in view of the recent reference to "amorphous polymorphism" [14], i.e. the existence of more than one distinct amorphous phase of the same substance. This phenomenon, which has been studied by computer simulation, evidently occurs in substances where the thermodynamic behaviour of the liquid state exhibits liquid-liquid phase separation or a tendency towards it.

In concluding this discussion of the methods of preparing polymorphs, several examples may be quoted of observations or procedures which, owing to their novelty or confinement to only one particular compound, cannot be

described as general. However, since systematic study of the factors which might be involved in effecting or affecting polymorphic transformation in these cases could in principle find wider application, brief mention of such examples is made here. The effect of explosion on polymorphs of chitin and chitosan has recently been studied [93]. Explosion of α-chitin resulted in no change, but explosion of hydrated chitosan of low crystallinity yielded a product with increased crystallinity as well as a small amount of the anhydrous phase. The effect of an electric field on polymorphic transformation in certain classes of compounds may be a phenomenon warranting systematic investigation. Strong temperature-dependence of the $\beta \rightarrow \alpha$ polymorphic transition of isotactic polypropylene (containing additives) has been observed when the system is exposed to an electric field [94]. The effects of neutron-irradiation on the kinetics of polymorphic phase transitions for several inorganic compounds have been reported [95], but similar studies using organic compounds as substrates are lacking. It has, however, been demonstrated that ionizing radiation can induce a polymorphic transformation in an organic compound. A polycrystalline sample of the β-polymorph of a diacetylene nitroxide has been reported to undergo transition to the α-polymorph on irradiation with CuKα or ^{60}Co sources [96]. X-ray powder diffraction was used to monitor this phase tranformation.

Finally, the role of serendipity in producing polymorphs may be mentioned. Two recent cases involved unsuccessful attempts to produce molecular complexes by reaction of two components in solution, resulting instead in the precipitation of crystals of one component in a desirable polymorphic form. Attempts to grow crystals of methotrexate by various techniques failed [97]. However, tetragonal crystals of the compound were obtained from a solution containing methotrexate and thymidine, prepared for the purpose of obtaining a co-crystal of these components. Similarly, attempted complexation between 5-sulphamethoxydiazine and p-aminosalicylic acid failed, the solution of these species in a 1:1 molar ratio producing instead large crystals of Form II of the sulphonamide [98]. Interestingly, this polymorph of the drug is the biologically most active form and is usually crystallized from ethanol followed by rapid cooling of the solution to -12 °C. This yields small particles with little or no geometrical form [99]. A possible explanation for the crystallization of Form II during attempts to produce the complex has been discussed [98].

3.2
Review of Investigative Methods

Having outlined the methods of preparation of polymorphs and pseudopolymorphs, this report now focuses on the methodology used to study these forms. The discussion commences with a survey of some well established and still widely used techniques, each of which is illustrated by one or more applications. This is followed by a survey of newer methodology which is being used to probe organic crystal polymorphism.

A wide spectrum of analytical techniques may be used to characterize polymorphs and pseudopolymorphs in terms of their structure, spectral energies, thermodynamic stabilities, kinetics of transformation and solubility behaviour.

The choice of analytical and physicochemical methods for the characterization of polymorphs is dictated by the need to measure properties which ultimately depend on the different internal arrangements of the same molecules in these phases. When pseudopolymorphs are also considered, the range of suitable analytical techniques is significantly broadened owing to the presence in the crystal of the solvating molecule and the possibility of analysing the physical and chemical changes which may accompany both formation and decomposition of pseudopolymorphs.

Some remarks on the use of white- and polarized-light microscopy serve as an appropriate introduction to this section. With the advent of more sophisticated analytical methods, the use of microscopy has, to some extent, fallen into neglect. This is unfortunate, since investment of relatively little time and effort in studying a crystal of a polymorph or pseudopolymorph by microscopy can be invaluable, enabling one to assess the overall quality of a recrystallization, to detect crystal faults, fractures and macroscopic inclusions, and obtain preliminary information which will facilitate subsequent X-ray examination. Detection of different crystal habits (acicular, tabular, bladed, plate-like, prismatic) using white-light microscopy is not necessarily indicative of polymorphism since crystal habit depends on crystallization conditions and may vary widely for a given polymorph. However, this method is useful for distinguishing polymorphs having different colours in reflected or transmitted light. Pseudopolymorphs which undergo pseudomorphosis (i.e. loss of solvent on removal from their mother liquor) tend to form opaque, microcrystalline masses which are also discernible by ordinary microscopy. Addition of a polarizing attachment allows distinction between optically isotropic crystals and anisotropic crystals as well as the measurement of refractive indices [100]. Optically isotropic crystals belong to the cubic system and have a single value for their refractive index. The vast majority of crystalline organic compounds are optically anisotropic, having multiple refractive indices and displaying numerous optical effects which may be used to differentiate polymorphic forms. Anisotropic crystals reveal themselves by producing variable interference colours as well as regular extinction of plane-polarized light on rotation of the microscope stage. They may be uniaxial (characterized by two principal refractive indices and belonging to the trigonal, tetragonal or hexagonal systems) or biaxial (with three refractive indices and belonging to the triclinic, monoclinic or orthorhombic systems). Measurement of these refractive indices is certainly a means of identifying a polymorph unequivocally, but is seldom done for this purpose. The uniaxial or biaxial nature of the crystal is easily determined from observation of the respective characteristic interference figure when the crystal is viewed with condensed (conoscopic) light. Taken together, extinction directions, crystal morphology and uniaxial or biaxial character can facilitate the identification of a new polymorph, as exemplified by the following case from our laboratory. Carbamazepine commonly crystallizes in the monoclinic system with a prismatic habit [101]. Microscopic examination of crystal batches obtained by recrystallization of the drug from a wide range of solvents confirmed the predominance of this form. However, crystals obtained from tetrahydrofuran were acicular, yielding extinction parallel to the needle-axis and presenting a uniaxial interference figure.

Measurement of the interfacial angles of a crystal section both microscopically and using an optical goniometer yielded values of 120 ± 1°. Thus, a new polymorph belonging to either of the trigonal or hexagonal systems was unequivocally identified. This was confirmed by subsequent X-ray photography which revealed a trigonal space group [102].

Owing to its vast depth of field, scanning electron microscopy (SEM) is widely used for observing the texture, morphology and surface features of both powders and large single crystals of polymorphs. The high vacuum used for sample observation precludes study of pseudopolymorphs containing volatile solvents but SEM micrographs of polymorphs are useful for purposes of identification provided that crystallization conditions and SEM sampling methods are carefully controlled.

Crystal density is an important technological parameter since it affects the flow properties of bulk solids. Due to the different bulk densities of polymorphs and their abilities to retain solvent, different isolation strategies are required in industry [24]. Furthermore, if mixtures of different solid phases are present in a sample (e.g. in a powdered pharmaceutical formulation), differences in component densities may lead to a heterogeneous product during processing due to phase segregation. Distinguishing polymorphs of the same compound by density measurement (determined by flotation or gas displacement pycnometry) is difficult because, as shown in Fig. 6b, differences in the densities of such species seldom exceed 5% and the experimental error of routine measurements is typically 2%. However, the latter can be reduced if special precautions are taken during flotation measurements, especially with regard to eliminating occluded air. Under these conditions, anomalously high or low measured densities may be useful indicators of the presence of pseudopolymorphs. Thus, e.g., a measured density of 1.30(1) g cm^{-3} for a crystal of doxylamine succinate obtained from ethyl acetate was sufficiently different from that of polymorphic Form I (ρ = 1.21(1) g cm^{-3}) to indicate the presence of a pseudopolymorph. Subsequent X-ray analysis showed the crystal to have the unexpected composition (doxylamine succinate)$_2$ · (succinic acid) [103].

Among the thermal methods of analysis, thermogravimetric analysis (TGA), differential thermal analysis (DTA) and differential scanning calorimetry (DSC) have been used extensively to quantify thermal events accompanying controlled heating of polymorphs and pseudopolymorphs [18, 23]. Figure 7 shows combined TGA and DSC traces for two pseudopolymorphs of nitrofurantoin [61], containing respectively *N,N*-dimethylformamide (DMF) and dimethylsulphoxide (DMSO).

In TGA, the sample (~5–10 mg) is heated at a predetermined rate and the weight is recorded as a function of temperature. This technique cannot distinguish polymorphs of a given organic compound, but for pseudopolymorphs which lose their included solvents prior to melting or decomposition of the parent ("host") compound, the percentage weight loss may be accurately measured and used to calculate the stoichiometry of the pseudopolymorph. Data recorded from the TGA traces shown in Fig. 7 indicated a nitrofurantoin:DMF stoichiometric ratio of 1:1 and a nitrofurantoin:DMSO ratio of 2:1. A TGA trace may reflect simple one-step weight loss of included solvent or more complex

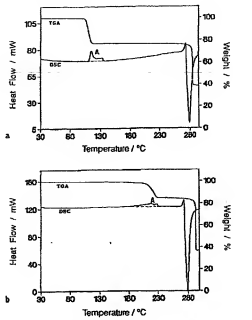


Fig. 7a, b. Combined TGA-DSC traces for pseudopolymorphs of nitrofurantoin containing: a DMF; b DMSO (Reprinted with permission from [61], copyright 1996, Gordon and Breach Publishers)

multi-step weight losses. Coupled with TGA, evolved gas analysis (EGA) using IR or mass spectrometry is an important means of identifying gaseous products [18] and may be used to quantify both the pyrolysis products from thermal degradation of polymorphs as well as solvents released from desolvation of pseudopolymorphs. Another important application of the TGA method is the determination of the activation energy for desolvation of a pseudopolymorph from traces recorded at varying heating rates [104]. Its application to pseudopolymorphs of succinylsulfathiazole [105] and tenoxicam [106], and to inclusion compounds of synthetic hosts [50] have recently been described.

In DTA, the sample temperature (T_s) is compared with that of a reference compound (T_r) as a function of increasing temperature. The resulting plot of ΔT ($=T_s-T_r$) vs T may display endothermic peaks corresponding to desolvation (for pseudopolymorphs) or fusion (for polymorphs) and exothermic peaks representing recrystallization or decomposition processes. In the related DSC technique, the difference in energy inputs into a compound and a reference substance is plotted against T during a controlled temperature programme. In

theory, the area under the curve in a DSC trace is directly proportional to the enthalpy change for the thermal event, but special precautions are necessary to obtain accurate values for this quantity [18]. An alternative method for determining enthalpies of desolvation of pseudopolymorphs, based on direct vapour pressure measurement of the liberated solvent as a function of temperature, has been described [50]. The DSC traces in Fig. 7 display endotherms (peaks A, above the baseline) whose temperature ranges correlate well with those of the TGA traces, confirming that these peaks represent desolvation processes. The bimodal nature of these endotherms is difficult to interpret but evidently reflects complex desolvation mechanisms. The difference in the DSC temperature ranges for loss of DMF (100–125 °C) and loss of DMSO (160–235 °C) is striking. These pseudopolymorphs were subsequently shown by single crystal X-ray analysis to contain their respective solvent guest molecules in constricted channels formed by the nitrofurantoin host framework; each DMF molecule is bound to one drug molecule by a single hydrogen bond, whereas each DMSO molecule forms two hydrogen bonds with neighbouring drug molecules, thus providing a possible explanation for the significantly higher onset temperature for the loss of DMSO. In this connection, the use of the simple parameter $T_{on} - T_b$ (T_{on} = extrapolated DSC desolvation onset temperature, T_b = boiling point of the guest solvent) has been proposed as a possible measure of the relative thermal stabilities of inclusion compounds [50]. For both pseudopolymorphs referred to in Fig. 7, desolvation produced the β-polymorph, as confirmed by the characteristic sharp fusion endotherm at 273 °C, followed by a decomposition exotherm peaking at 279 °C.

Recent reviews of thermoanalytical methods and their application to polymorphic systems [18, 23] include descriptions of modern instrumentation, discussions of the influence of instrumental and sample parameters on measured data, as well as guides to the interpretation of DSC traces. One review [23] includes a summary of the thermodynamic rules established by Burger [107] for distinguishing monotropic and enantiotropic transitions. It is worth emphasising that in the absence of supporting techniques such as hot stage microscopy (HSM), X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FT-IR), errors in the interpretation of DSC data may result. Reference to HSM was made earlier in connection with its use in screening recrystallized batches of a compound to identify polymorphs and pseudopolymorphs. Apart from its ability to yield melting points and transition temperatures for polymorphic changes or glass transitions, HSM is an invaluable adjunct to the DSC technique in that visual recording of thermal events (e.g. desolvation, recrystallization, sublimation) may corroborate assignments of endothermic or exothermic peaks appearing in DSC traces. As pointed out recently [108], visual observation can sometimes be more sensitive indicators of phase changes than DSC measurements. The report describes the construction and use of the device shown in Fig. 8, which allows simultaneous DSC measurement and microscopic observations with a video-camera. A second camera is used to give a direct read-out of the varying sample temperature.

Solid-state infrared (IR) spectroscopy in the spectral range 400–4000 cm^{-1} has been used extensively to distinguish different polymorphic forms as well as

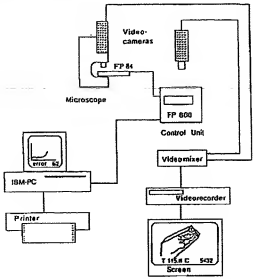


Fig. 8. Schematic description of an apparatus for simultaneous visual and DSC observations using the Mettler Thermosystem FP800 (Reprinted with permission from [108])

different pseudopolymorphs of the same compound. To ensure transparency to IR radiation, samples are usually prepared by trituration of crystalline material and suspension in Nujol or by pressing a co-ground mixture of the compound with KBr into a disk. However, since complete polymorphic transformation can sometimes be effected even by gentle manual grinding, it is advisable to confirm the existence of polymorphs by an independent method prior to a detailed IR analysis. Differences in molecular conformation and crystal packing features for different polymorphs of an organic compound manifest themselves in IR spectra chiefly by frequency shifts and splittings in, e.g., N-H, O-H and C=O absorption peaks (when these functional groups are present) due to differences in hydrogen bonding arrangements. The goal of IR analysis is the deduction of polymorphic structural differences from such spectral effects, but detailed interpretation is often limited and it is somewhat easier to rationalise these effects retrospectively if single crystal X-ray data can be obtained for the various forms.

This is illustrated by the following examples from our laboratory. A study of the literature on the compound sulphamerazine revealed that its polymorphism was controversial. The crystal structure of the drug recrystallized from acetone had been shown to be orthorhombic, Pbca with Z=8 [109]. Centrosymmetric hydrogen bonded (N-H···N) dimers exist in the crystal structure as shown

schematically in Fig. 9. We obtained crystals of a second polymorph of sulphamerazine [110] by recrystallization from methanol and established the space group as $Pn2_1a$, $Z=8$. This form contains the same hydrogen bonded motif as shown in Fig. 9, but it is pseudo-centrosymmetric. The two polymorphs are distinguishable from IR spectra, the form containing the centrosymmetric dimer (with only one unique N-H···N hydrogen bond) yielding only one IR peak (3453 cm^{-1}) assignable to $\nu_{as}NH$ whereas that containing the pseudo-centrosymmetric dimer (with two distinct N-H···N hydrogen bonds) displayed a doublet (3493, 3478 cm^{-1}).

We recently reported FT-IR spectral data for seven crystalline forms of the androgen dehydroepiandrosterone [111], showing that differences in both fine structure and in the locations and intensities of major absorption bands can be used to distinguish them. X-ray crystal structures of three forms were determined [112], Form I (a polymorph), Form S1 (a 4:1 hydrate) and Form S4 (a methanol half-solvate). Form I (space group $P2_1$, $Z=4$) contains infinite chains of steroid molecules linked head-to-tail (shown schematically in Fig. 10) by two crystallographically distinct intermolecular O-H···O=C hydrogen bonds, consistent with two observed O-H stretching bands (3501, 3458 cm^{-1}). Doublets for the O-H absorption bands observed also in both Forms S1 and S4 can be correlated with distinct O-H···O hydrogen bonds observed by X-ray analysis, and specifically, with unusual "flip-flop" hydrogen bonding in Form S4. In this study, attempts to determine the thermodynamic relationships between the various polymorphic forms were based partly on the application of Burger's IR rule [107] which relates IR band positions to relative stabilities.

The use of IR-spectra to distinguish polymorphs from pseudopolymorphs of the same parent compound, or to distinguish pseudopolymorphs containing different solvents, is relatively straightforward, since solvent molecules which are commonly incorporated in pseudopolymorphic crystals (e.g., water, ketones, alcohols) will exhibit their own characteristic absorption bands. IR spectroscopy was used to distinguish polymorphs of the antidiabetic drug glibenclamide from its solvates with pentanol and toluene [113]. Pseudopolymorphs of the

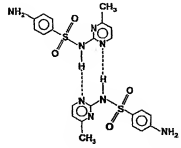


Fig. 9. Schematic representation of the hydrogen bonded dimer occurring in two polymorphs of sulphamerazine

compound gossypol (a toxic constituent of cotton-seed) obtained from three different solvents showed characteristic differences in their IR spectra in the 3500 cm^{-1} region [114]. Pseudopolymorphs of a host containing the same guest in different molar ratios are also known. An alicyclic diol of the helical tubuland family [115] forms two crystallographically distinct lattice inclusion complexes with 1,2-dichlorobenzene having host-guest ratios of 4:1 and 3:1 [116]. These were readily distinguished from their IR spectra in the fingerprint region.

The spectral techniques available for the characterization of polymorphs and solvates have been reviewed [27] and the merits of FT-IR spectroscopy for the acquisition of high-quality spectra have been stressed. The review also lists drug substances whose polymorphism has been investigated by IR-spectroscopy. Another recent review on the use of the IR method to investigate polymorphism is based on spectra for a derivative of sulphadoxine as a model compound [21]. Dynamic aspects of polymorphism are also amenable to study by IR spectroscopy. The effect of hydrogen bonding on the lattice dynamics, polymorphism, mesomorphism and phase tranformations of long-chain carboxylic acids as gauged from IR spectra has recently been reported [117].

A relatively new trend is the application of near-IR spectroscopy for compound identification and quantitation in many industrial environments including chemical processing and food science. The advantages of near-IR over conventional IR spectroscopy for the investigation of polymorphs have been described [118]. A fast, sensitive pattern recognition method for identifying a polymorph and determining its quality using near-IR spectra has been developed [119]. Pattern recognition methods involve training a computer to recognize spectra of acceptable samples of a material and to reject unacceptable ones by multivariate statistical analyses. This study showed that the method described can discriminate between the desired polymorph and other crystalline forms, the statistical results indicating that levels as low as 2% of an adulterating polymorph may be reliably detected. The authors maintain that the near-IR method, which can be automated, provides a reliability comparable to that of XRD for polymorphic identification but is likely to be less costly to implement.

There is a growing interest in the use of Raman spectroscopy for studying polymorphism and pseudopolymorphism [27]. The Raman technique has recently been used to distinguish two triclinic polymorphs of terephthalic acid [120] and, in combination with IR spectroscopy, to study the conformational polymorphism of 1-bromopentane [121].

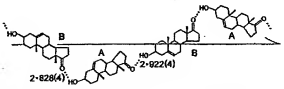


Fig. 18. Head-to-tail hydrogen bonding in Form 1 of dehydroepiandrosterone. A and B are crystallographically distinct and distances are in Å

The use of white-light microscopy to identify differently coloured polymorphs was mentioned above. Colour polymorphism may be quantified using UV/vis electronic spectroscopy (diffuse reflectance, fluorescence methods). Colour differences for polymorphs of the same compound may originate from differences in charge transfer interactions or different molecular conformations in the crystals. Pseudopolymorphs may also be distinguished using fluorescence spectroscopy since they may display characteristic absorption maxima as well as fluorescence intensities. Systems which exhibit colour polymorphism have been reviewed [27].

Implicit in the examples cited above to illustrate applications of various analytical methods for studying polymorphism is the definitive role of single crystal X-ray diffraction in providing unequivocal evidence for the very existence of these forms. Different polymorphs of a given compound have, by definition, non-equivalent crystal structures and the method of choice for detecting such non-equivalence is single crystal X-ray analysis. In many instances, the differences between polymorphs are so subtle that they can be distinguished only by this method [79]. This technique goes well beyond merely detecting different forms, providing in addition a wealth of structural information for each species at a level of detail and precision currently unsurpassed by other analytical techniques. Furthermore, as illustrated by several of the examples above, detailed knowledge of the molecular parameters and crystal packing features obtained by this method provides a very reassuring basis for the interpretation of results gleaned from other analytical techniques. For these reasons, much effort is expended by researchers in growing single crystals of polymorphs and pseudopolymorphs with adequate quality for complete X-ray structural elucidation.

For routine crystallographic studies of polymorphs or pseudopolymorphs containing small and medium-sized molecules, the methodology of crystal structure determination is well established and the reader is referred to standard monographs on the subject [122, 123]. However, a few points on current routine methodology are worth emphasising in order to place some of the newer developments (to be discussed later) in perspective.

Existing standard methods employ single crystals usually no smaller than 0.1–0.5 mm, automated X-ray intensity data-collection (typically with a four-circle diffractometer) and standard crystallographic software packages for data-processing, structure solution and refinement. Room-temperature data-collection is common, despite the gain in structural definition possible with cryostatic devices for cooling the crystal. Low-temperature analyses are sometimes carried out on pseudopolymorphs in which the included solvent molecules tend to have excessive thermal motion or are disordered at room-temperature. The advent of fast detectors (organic scintillators, image-plates, multiwire area detectors) and more powerful structure determination packages has greatly increased the speed with which crystal structures can be solved and refined [124].

In the absence of single crystals of suspected polymorphic forms, X-ray powder diffraction (XDP) serves as the primary test for non-equivalence of crystal structures, the XDP pattern of such a species being unique [25]. However, since

sample preparation involves grinding in a mortar to an average particle size ≤100 μm to minimise preferred orientation effects, the possibility of a pressure-induced phase transformation during sample preparation must be considered. The XRD pattern of an unground sample of the material may be checked as a precautionary measure. Patterns are generally recorded on samples with mass range 200–500 mg using automatic diffractometers with strip chart output. The method is generally non-destructive, allowing recovery of the sample. Film methods (e.g. the Debye-Scherrer technique) require a few mg of material only and are still frequently used when only small amounts of polymorphic forms can be isolated. According to the guidelines specified in the US Pharmacopeia [125], agreement between a sample and a reference standard should be within the calibrated precision of the diffractometer for diffraction angle (typically a reproducibility of ± 0.10–0.20° in 2θ) with permissible relative intensity variations of up to 20%.

A recent analytical study stresses the growing need, prompted partly by legislatory requirements, to differentiate polymorphs and to quantify polymorphic mixtures in pharmaceutical production [126]. The compounds benzil and benzoic acid were chosen as a model system for the development of an XRD protocol which could be extended to the quantification of mixtures of drug polymorphs. The study involved the evaluation of sample thickness, the determination of preferred orientation effects, optimum milling conditions and the construction of diffraction intensity-composition calibration curves for mixtures of benzil and benzoic acid. Since the composition of such mixtures can be accurately determined by an independent method, namely HPLC, validation of the quantification of mixtures by the XRD protocol was possible. It was concluded that the protocol is accurate for the model system to within a few percent. It is desirable that the general validity of the approach suggested be tested on a range of real polymorphic systems.

The information contained in an XRD pattern of a powdered material is a significantly condensed version of that obtained by single crystal X-ray analysis and it is a routine matter to reconstruct the XRD pattern of a polymorph by computation, using as input the space group data, refined atomic coordinates, atomic thermal parameters and unit cell data which have been obtained from a single crystal X-ray study of that species. Some years ago, it was recommended [127] that computation of the XRD pattern of a new polymorph should always be carried out if single crystal data become available. The computed pattern, being free of experimental aberrations, serves as the best reference pattern for the polymorph in question. We have followed this practice and can strongly endorse its usefulness, not only for the purpose of identifying the polymorphic form present in a newly crystallized sample, but also for assessing the polymorphic purity of such a sample. Figure 11 shows the experimental XRD pattern of a polymorph of the non-steroidal anti-inflammatory drug tenoxicam and, for comparison, the computed pattern based on the single crystal X-ray data [106]. The level of agreement in peak positions attests to the polymorphic purity of the sample (no extraneous peaks being evident in the experimental pattern) while the discrepancies in corresponding peak intensities indicate some degree of preferred orientation in the sample. Finally, since the sample was a powder derived

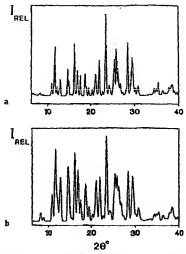


Fig. 11a, b. XRD patterns for a polymorph of tenoxicam: a experimental; b calculated from single crystal data. (Reprinted with permission from [106], copyright 1995, American Chemical Society and American Pharmaceutical Association)

from large single crystals previously identified by X-ray analysis, the overall agreement of the patterns reveals the non-trivial result that grinding single crystals of the polymorph does not effect a phase transformation to another polymorph. In the case of the drug probucol (Fig. 3), the experimental XRD patterns for the two polymorphs were identical as a result of transformation of one polymorph into the other during sample preparation [59].

Neutron and electron diffraction techniques have also been used to some extent to study the polymorphism of organic substances. Owing to its ability to locate hydrogen atoms accurately in crystals, neutron diffraction is the method of choice for distinguishing subtle differences in hydrogen bonding for polymorphic crystal structures. However, the technique is costly and the low intensity of neutron beams demands relatively large single crystals (around 1 mm^3 or larger) which are seldom available. Crystallographic studies are therefore usually carried out on polycrystalline samples, yielding powder neutron diffraction patterns analogous to XRD patterns. A fairly recent development is the use of pulsed neutron sources coupled with time-of-flight analysis [100]. As an example of its application to the study of polymorphism, the case of cyclohexanol maybe cited [128]. Structural elucidation of three polymorphs (cubic (I), monoclinic (II) and orthorhombic (III)) revealed that I is disordered, that II contains a cyclic dimer of cyclohexanol, and that a polymeric arrangement exists in III.

Because incident electrons interact strongly with crystals, electron diffraction is capable of yielding detailed structural information from very small crystals

(typically 0.01 – 0.02 mm). It has been cited as a more reliable method for determining crystal unit cell and space group data than computer-assisted indexing of XRD patterns [100]. The crystal structure of a high-temperature polymorph of chitosan from electron diffraction data has recently been reported [129]. Structural modelling of this complex molecule involved constrained linked-atom least-squares refinement with stereochemical restraints.

Dilatometric analysis is a less widely used technique for characterising polymorphs, but has, for example, been applied to the study of polymorphic transformations occurring in theobroma oil, methyl stearate and chloramphenicol [25]. Substances which contract as they transform from a metastable (less dense) polymorph to a stable (more dense) polymorph can be studied by measuring their specific volume as a function of temperature. A recent study involved the combined use of dilatometry and neutron scattering to characterise the orthorhombic and monoclinic polymorphs of *m*-nitrophenol [130]. Crystals of both polymorphs showed significant anisotropy of expansion and it was possible to reconcile the direction of lowest expansion with that of the hydrogen bonding interactions. Attempts to correlate these results with those obtained from IR and Raman spectra were subsequently reported [131].

Solubility and dissolution rate analyses are of vital importance for polymorphs and pseudopolymorphs of pharmaceutical relevance. For a given drug, metastable polymorphs tend to have higher solubilities and faster dissolution rates than the stable polymorph. When metastable forms are employed in solid dosage forms (tablets, capsules), they generally yield higher and earlier blood serum levels [25]. Thus, for potent drugs with a narrow therapeutic index (e.g. the cardiotonic digoxin), inadvertent use of a metastable polymorph in a tablet could result in patient death from overdose. In vitro dissolution testing is therefore carried out routinely as part of the quality control of manufactured tablets and capsules. It may also be performed directly on powders or single crystals of the drug polymorphs or pseudopolymorphs comprising the active agent. Essentially, this involves placing the sample in the dissolution fluid, agitating it in a reproducible manner at constant temperature (usually 37°C), and measuring the drug concentration in solution as a function of time. Compendial testing methods include the rotating basket, rotating paddle, and flow-through cell techniques. Automated systems employ a peristaltic pump which circulates the dissolution fluid through a UV spectrophotometer flow-cell for continuous monitoring of drug concentration. The advantages and disadvantages of modern analytical techniques available for dissolution testing have been reviewed recently [22].

Polymorphs generally dissolve more rapidly than their hydrates, but there have been reports of drug pseudopolymorphs containing, e.g., ethyl acetate or *n*-pentanol, which display enhanced solubilities, both in vitro and in vivo, when compared with their nonsolvated forms [25]. Figure 12 shows powder dissolution rate curves for two polymorphs of nitrofurantoin. The significance of the curves for this system is that the retarded initial dissolution rate for the α-polymorph may render it more favourable for pharmaceutical formulation since there is evidence that adverse side effects may be associated with rapid absorption of the β-polymorph [61].

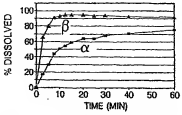


Fig. 12. Powder dissolution rate curves for two nitrofurantoin polymorphs. (Reprinted with permission from [61], copyright 1996, Gordon and Breach Publishers)

Measurements of the equilibrium solubilities of a polymorph as a function of temperature permit evaluation of the enthalpy of dissolution from a van't Hoff plot (i.e. logarithm of solubility vs T^{-1}). The point at which such plots intersect for two different polymorphs of the same compound corresponds to the transition temperature for their interconversion.

It should be stressed that the analytical methods surveyed above achieve their optimum effectiveness and lead to deeper insights into polymorphism and pseudopolymorphism when used in combination. A simple illustration is the combined use of dissolution rate analysis and crystal structure analysis of two polymorphs. Used individually, these techniques would produce merely data, whereas the combined approach could lead to an explanation for the differences in dissolution rates based on differences in crystal packing. Some reported studies utilise a wide range of techniques, permitting a comprehensive analysis of a polymorphic system, while others may use only two, but nevertheless effective, complementary techniques (e.g. DSC and XRD). The following examples selected from the recent literature illustrate this point.

Four polymorphic forms (I-IV) of the drug diflunisal (analgesic, anti-inflammatory) were prepared and characterised by DSC, HSM, IR, XRD, and dissolution studies [132]. The thermoanalytical methods revealed that on heating, all polymorphs recrystallized to the stable one (Form I) prior to melting. Only one transition peak in the DSC traces was observed, for the transformation of Form III to Form I. Intermolecular hydrogen bonding features were deduced from IR spectroscopy and this method was also shown to distinguish all the forms. Measurements of the intrinsic dissolution rates indicated (unexpectedly) that Forms II, III and IV had slightly lower values than that of the stable form. Results of this kind are invaluable for ensuring correct formulation of the drug. Some examples of other important drug polymorphic systems for which similar, fairly comprehensive studies have recently been reported include acyclovir [133], meprobamate [134], prasterone [111], ranitidine hydrochloride [135], and chloramphenicol palmitate [136].

Other compounds whose polymorphism and/or pseudopolymorphism have been investigated in recent years, together with the methods used, include pentachloropyridine (DSC, Raman and IR spectroscopy) [137], cyclohexanol (far-IR, adiabatic calorimetry) [138], nifedipine (HSM, IR, DSC) [139], sulfanil-

amide (DSC, XRD) [140], paraffins $C_{24} - C_{60}$ (DTA, thermobarometric analysis) [141] and tripalmitin (DSC, thermodiffractometry, microcalorimetry) [142]. This list represents a very small selection, merely serving to indicate the variety of techniques employed.

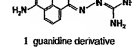
Several recent developments in the areas of structure determination are having a significant impact on the study of organic crystal polymorphism and these are now surveyed, together with examples of applications to specific systems. In the discussion above, it has been maintained that a knowledge of the crystal structure of a polymorph provides a sound basis for interpretation of other analytical data pertaining to that species and is crucial for even a rudimentary understanding of phase transformations at the molecular level. It is also well known that many polymorphs of technological interest can not readily be obtained as single crystals suitable for X-ray structural elucidation. However, since the advent of the Rietveld, or "whole-pattern", refinement technique [143] which allows crystal structure refinement using X-ray or neutron powder diffraction data, the status of powder XRD has been elevated from that of a mere "fingerprinting" technique to one of formidable power as a structure-solving tool. This power has been significantly enhanced by recent progress in obtaining trial structural models for Rietveld refinement directly from the diffraction intensities. Since the powder pattern is a one-dimensional projection (on 2θ) of the three-dimensional single crystal diffraction data, a key problem in this challenging research area is unravelling the intensities of severely, or exactly, overlapping reflections. In a recent study, an algorithm for achieving this was reported [144] and, together with software routines for space group determination and a fast iterative Patterson squaring routine, this combination led to the successful *ab initio* crystal structure determination of an aluminophosphate-based molecular sieve from X-ray powder data exclusively. It is noteworthy that as many as 65% of the reflection data used in this analysis suffered from severe overlap. This approach holds great promise for future structure determination of polymorphs which hitherto have been obtained in the form of powders only.

More recently, a method using powder diffraction for structure solution was described in which structural information is not extracted directly from the intensities; instead, trial structures are generated using a Monte Carlo approach [145] commencing with a collection of atoms initially randomly placed in the crystal unit cell. A series of trial structures is then generated by random movement of the atoms, each trial structure being accepted or rejected depending on the level of agreement between the calculated and experimental powder patterns. The Monte Carlo method employed is based on the Metropolis importance sampling algorithm using the crystallographic R-factor as the basis for constructing trial models. The best model is then used in a conventional Rietveld refinement. The structures of two compounds, with eleven and twelve non-hydrogen atoms in the respective asymmetric units, were solved by this procedure using high-resolution powder X-ray intensity data measured with a position-sensitive detector. The novel feature of this method is the use of the Monte Carlo algorithm for generating trial structures.

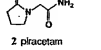
Crystal structure prediction based on lattice energy minimisation has already been discussed and attention has been drawn to the serious drawbacks of this

approach when used on its own. However, used in combination with the Rietveld method, its chances of success are very significantly improved, as recently demonstrated for a polymorphic system [146]. Of two polymorphs, A and B, of a guanidine derivative (1), only form B was available in the form of single crystals and its structure was solved in the conventional manner using X-ray diffraction methods. Polymorph A was available only as a powder and the structural problem was further complicated by molecular features allowing not only for different conformers of the molecule, but also different tautomers. Indexing of 26 lines of an XRD pattern of polymorph A measured on a Guinier camera led to the assignment of the triclinic system. Energy calculations using MOPAC93 were used to search for likely conformers and tautomers suitable for crystal packing calculations. High-precision ab initio quantum mechanical methods were used to confirm the trial models, the four most stable ones being chosen for packing analysis using a corrected Dreiding force-field. Two routes were pursued, one assuming the XRD indexing results, the other ignoring them. In the first case, crystal structures were generated for the space group P1 only, leading to a most stable structure with unit cell parameters very close to those deduced from the indexing. Subsequent optimisation and Rietveld refinement yielded a final structure with an R-factor of around 10%. When the indexing results were ignored, stable conformers were packed in common space groups (e.g. $P\bar{1}$, $P2_1/c$, $P2_12_12_1$). Of the predicted force-field minimised structures, that which gave the best agreement with the experimental XRD pattern turned out to be one in the space group $P\bar{1}$. Following interactive Rietveld refinement, this structure (gratifyingly) converged to the same one as determined assuming the indexing results. A very important feature of this study was the use of an ordinary in-house XRD pattern (as opposed to a high-resolution pattern) for the indexing and Rietveld refinement steps. The authors pointed out that both the ab initio quantum mechanical as well as the packing calculations for this problem consumed enormous amounts of computational resources. They nevertheless predicted that structure determination based on an ordinary powder pattern and packing calculations could evolve into a reliable and inexpensive routine procedure in view of the envisaged drop in computational costs which they state is about an order of magnitude every three years.

The structure of a metastable and shortlived polymorph of the drug piracetam (2) has been solved from XRD data using the AAP method [147]. This species (Form I) was prepared by heating a mixture of Forms II and III and quenching at room temperature. Form I spontaneously transforms into II at 298 K within a few hours of quenching, but it was possible to capture high-resolution XRD data within two hours using a position-sensitive detector. The same (known) molecular conformation as occurs in Forms II and III



1 guanidine derivative



2 piracetam

was assumed in the crystal packing calculations and the AAP method yielded two distinct minima, only the higher energy structure being correct (consistent with the metastability of Form I) as shown by successful Rietveld refinement. This impressive study supports the contention that the need for single crystals for structure solution is becoming less critical [65] and also demonstrates that even shortlived organic polymorphic species can be structurally characterised.

Another major development which continues to have a significant impact on structure determination of polymorphs has been the advent of synchrotron radiation as a source for diffraction studies [148]. This radiation, which is tuneable and whose intensity exceeds that of conventional X-ray sources by orders of magnitude, is bringing about a revolution in structure determination, not only for polymorphs, but for materials in general. In particular, very small crystals (in the micron range) can now be analysed using data generated using synchrotron radiation and collected with ultra-rapid detection devices. Time-resolved studies are also amenable to study with synchrotron radiation owing to its pulsed time structure and the possibility of recording the X-ray diffraction data from picosecond exposures. An on-line system using in situ X-ray diffraction has recently been developed for examination of the polymorphic forms of pharmaceutical materials [149]. It comprises a temperature-controlled stainless steel in situ cell for X-ray measurements attached to a two-circle diffractometer on a beamline at the Daresbury Synchrotron Radiation Source. The pH of a solution of a drug is varied by titration in a glass solution-saturation vessel and crystallization due to change in the solute solubility yields a crystallized slurry which is circulated through the cell for continuous monitoring of its diffraction pattern.

For the (unnamed) antibiotic investigated, Fig. 13 shows a series of diffraction patterns measured at different temperatures. From these, the transformation between two solvates of the drug at around 309 K is clearly evident. Using the system described, enthalpy release following crystallization can also be measured by monitoring the temperature of the crystallization solution. The authors point out the growing awareness of the importance of understanding crystallization processes for many specialty chemicals in process technology and the increasing popularity of on-line characterization techniques. In situ synchrotron X-ray diffraction offers the advantages of non-invasive characterization of polymorphism and polymorphic changes, and the possibility of studying very small crystalline particles.

The investigation of polymorphism and pseudopolymorphism by means of solid-state NMR spectroscopy is also gaining in popularity owing to recent advances in both instrumentation and computer pulse sequence strategies [27]. Most studies employ ^{13}C or ^{15}N NMR since ^{1}H NMR produces broad, uninformative peaks even after removal of proton-proton dipolar interactions. In the MAS ('magic angle spinning') technique, the sample is spun at high velocity at the critical angle of 54.74° with respect to the applied magnetic field. The resulting averaging of the chemical shift anisotropy reduces spectral broadening considerably, yielding sharp peaks with fine structure from which valuable information on individual atomic chemical environments can be deduced. To

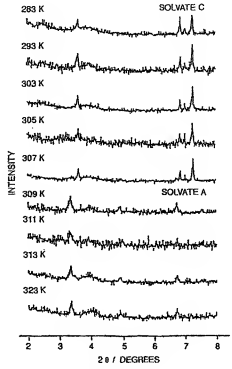


Fig. 13. In situ X-ray diffraction patterns of an antibiotic substance for crystallization range 283–323 K. (Reprinted with permission from [149])

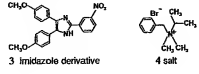
accelerate data-acquisition, enhancement of ^{13}C magnetisation is achieved by the CP ("cross polarization") strategy and the combined method is referred to as CPMAS NMR spectroscopy. With it, polymorphic conformational differences, crystal packing differences and the effects of hydrogen bonding may be characterised. Varying the sample temperature allows dynamic studies of solid-solid phase transformations. Incorporation of solvents in pseudopolymorphs introduces significant changes in local environmental magnetic effects through, e.g., hydrogen bonding, and such changes are therefore also open to study by the CPMAS technique.

Figure 14 shows ^{15}N CPMAS spectra for four polymorphic modifications [150] of the imidazole derivative (3), from which it is clear that ^{15}N chemical shifts are good indices for their identification. ^{13}C CPMAS has also recently been used to distinguish three polymorphs of complex triacylglycerols [151]. Spectral

Fig. 14. ^{15}N CPMAS spectra for four polymorphs of the imidazole derivative 3. (Reprinted with permission from [150], copyright 1996, Gordon and Breach Publishers)

differences were used to characterise conformations and crystal packing differences in the three forms. Examples of applications of CPMAS to the study of polymorphism and pseudopolymorphism of pure drug substances [27] as well as drug-excipient mixtures [152] have been described.

In a recent study of the molecular motion in the crystal of the salt (4), the results of ^{13}C CPMAS NMR and high-resolution powder XRD were combined



3 Imidazole derivative 4 salt

[153]. An important feature of this study was the determination of the activation energy for rotation of one of the N-methyl groups in the crystal from variable-temperature NMR data. The X-ray crystal structure was solved from high-resolution powder XRD data using direct phasing methods and refined by the Rietveld method. It was possible to reconcile the NMR-derived activation energy with the crystal packing which revealed that one of the N-methyl groups is significantly more hindered than the other. This study illustrates the potential of a combined approach to probing molecular motion in the solid-state. Its application to polymorphic systems is an obvious and desirable extension.

3.3
Polymorphic Systems and Polymorphic Changes – Case Studies

Four selected case studies are cited here to illustrate some of the difficulties encountered in research on organic crystal polymorphism, even when relatively simple molecules are involved. Since space does not permit discussion, précis of these model studies are presented in the hope that the interested reader will consult the original, absorbing accounts for details.

The polymorphism of anthranilic acid has been studied for over a century. Despite this, many properties of crystals of this simple molecule remain obscure and a recent re-examination of the phase transitions was undertaken using X-ray and FT-IR techniques [154]. A special feature of this system is that phase transitions appear to depend subtly on grinding pressure. The main conclusion drawn from the investigation cited is that polymorph I is transformed into polymorph III on heating, not into polymorph II as commonly stated in the literature.

The crystallization behaviour of the high explosive 2,4,6-trinitrotoluene (TNT) has invoked intensive research since the first report on its optical investigation in 1879 [155]. Despite numerous on-going studies, the conditions for crystallizing the monoclinic and orthorhombic forms lack definition. A systematic re-examination using Laue and Weissenberg X-ray methods, DSC, goniometry and computer simulations was undertaken to establish the role of crystallization conditions in determining the nature of polymorphism as well as TNT crystal morphology [156]. Complicating features of this system are extensive twinning in the monoclinic phase and remarkable similarity in the structures of the two polymorphs which results in their XRD patterns being almost indistinguishable. The study established (inter alia) conditions for crystallizing the two forms, finding that their occurrence was determined by growth solvent only and was independent of the rate of crystallization. The orthorhombic polymorph was shown to be metastable relative to the monoclinic polymorph.

Terephthalic acid (TA) is an important industrial chemical used in the production of polyester. Its purification involves multi-stage recrystallization under extreme conditions. The phase characterisation and morphologies of Forms I and II of TA were studied by HSM, thermal analysis, Raman spectroscopy, single crystal Laue X-ray diffraction, lattice energy and morphological calculations [157]. Contrary to earlier reports, this study revealed that Form II (not Form I) is the thermodynamically stable form of TA under ambient conditions. The apparent stability of Form I was shown to be due to twinning in microcrystals

leading to "collective stabilisation", which prevents spontaneous transformation to Form II.

Structural, thermodynamic, kinetic and mechanistic aspects of phase transformations among three differently coloured forms of dimethyl 3,6-dichloro-2,5-dihydroxyterephthalate have been studied by various methods [108] including simultaneous DSC and optical microscopy using the apparatus shown in Fig. 8. The three forms, (Y) yellow, (LY) light yellow and (W) white, which exhibit extraordinary polymorphic behaviour, are now known to be conformational polymorphs and the transformations among them appear to occur by nucleation and growth of the new phase outside the domains of the old one, not by single-crystal to single-crystal transformation. Some perspective on research on polymorphism can be gained from the concluding remarks of this account which stress the prevailing lack of understanding of the complexity of phase transitions and the formidable experimental difficulties experienced in obtaining reproducible results.

4
Towards Control of Polymorphism

4.1
The Need for Polymorphic Control

In a review sketching the important developments in synthetic organic chemistry during the past 25 years and predicting future directions [158], it was emphasised that the exciting synthetic targets today are no longer molecules to be prepared "for their own sake", but rather, they are systems possessing specific functions or properties. The systems which are being pursued with this goal in mind are largely those whose properties are governed by non-covalent interactions, as evidenced by the rapid growth of the discipline of supramolecular chemistry in recent years. In the realm of solid-state chemistry, the goals of crystal engineering [3] are, analogously, the design and preparation of materials with specific properties (e.g. metallic conductivity, thermochromism, photoactivity, second-harmonic generation), these properties being strictly dictated by the crystalline assembly or supramolecular organization in the solid-state. Preparation of such assemblies has been severely impeded by the lack of an underlying theoretical framework for understanding the subtle interplay of factors determining crystal packing. There is no guarantee, in the first instance, that a given molecule will form a stable crystal which will survive the treatment to which it will be subjected. The occurrence of polymorphism (in which both thermodynamics and kinetics play a role) is an additional complicating factor, since the three-dimensional array possessing the desired property is but one, unique arrangement among many alternative arrays having similar free energies, but lacking that property. As an example, one property of solid materials currently attracting widespread attention is that of non-linear optical (NLO) behaviour, whose effect is second-harmonic generation (SHG). This property has potential for the design of laser devices, optical communications and information processing. SHG depends on both the molecular hyperpolarizability

(β) of the compound in question and on the orientation of the molecules in the crystal. Specifically, a non-centrosymmetric packing arrangement is required for a solid to exhibit NLO properties and, while it is possible to alter the value of β by synthetic modification, encouraging molecules to adopt a non-centrosymmetric packing arrangement is a formidable challenge.

The question of whether polymorphism is the "nemesis of crystal design" was discussed at length nearly a decade ago [3]. Since then, however, systematic studies in the areas of crystal nucleation, growth and morphology have led to definite proposals for rational stereospecific control of polymorphism as well as some success in its attainment in special cases [36]. Thus, while ab initio crystal structure prediction is still a distant goal [72] and the preparation of specific, desired phases is still elusive, there is at least the possibility of rational intervention during the nucleation or growth stages of crystallization for influencing the outcome, provided that the detailed polymorphic behaviour of the system has been established from systematic study. This again provides strong justification for the application of a wide range of techniques for comprehensive studies of systems exhibiting polymorphism. As implied by case studies described earlier in this report, there are numerous instances in industrial environments where control of both crystal morphology and the polymorphic form of a compound is vital, and here the outlook for success, based on the strategies to be described briefly below, is promising.

4.2
Current Strategies and Prognosis for Crystal Engineering

Practical "control" of the polymorphic outcome of a crystallization process can be interpreted at different levels, ranging from the purely empirical, such as the use of identical crystallization conditions in the hope of obtaining a particular polymorph in a reproducible manner, to a much more ambitious strategy based on, e.g., rational design of additives which could suppress or inhibit nucleation of undesired polymorphs through selective molecular recognition mechanisms, thus allowing unhindered growth of a desired metastable polymorph. Between these extremes, there are different variations, both physical and chemical, that are effective in producing specific polymorphs, as exemplified below. Reports of this kind appear in the recent patent literature, attesting to the technological and commercial importance of the polymorphs concerned. A patent for the preparation of Form 1 of the widely used antiulcerative drug ranitidine hydrochloride describes its crystallization from a mixed solvent in the presence of seed crystals, the preferred process involving in situ reaction of HCl with the free-base [159]. Industrial-scale preparation of the stable polymorphic form of *N*-(4-hydroxyphenyl)retinamide is effected by successive chemical reactions with all-*trans* retinoic acid as the starting material [160]. The desired polymorph is formed by recrystallizing the wet product from 95% EtOH. The β-form of an organic phosphite, reported to be an effective process stabiliser for polyolefins, has been prepared by heating the compound at 125°C in the absence of solvent [161].

Desolvation of pseudopolymorphs may be considered as a means of polymorphic control. This was discussed earlier in this report in the context of

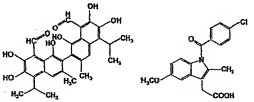
altering the rheological properties of drug polymorphs in particular. However, thermal treatment or vacuum drying has also been applied to more general inclusion compounds containing both natural and synthetic host molecules in order to prepare pure polymorphic forms of the host. Of the seven polymorphic forms of gossypol (5) identified thus far [162], only two were obtained by appropriate recrystallizations from solution, while the remainder resulted from desolvation of various gossypol clathrates. The latter could be divided into isostructural groups and it was found that all members of a given group yielded the same polymorph on desolvation. These unusual and intriguing results imply the existence of a "template" effect in this system. Similar experiments with pseudopolymorphs of indomethacin (6) also yielded a series of isostructural pseudopolymorphs [83], but in this case there was virtually no correlation between a given series and the identity of the polymorph resulting from desolvation. A systematic study of such systems to elucidate the mechanisms of desolvation is desirable if this process is to be exploited in a predictive way for the control of polymorphism.

Polymorphic inclusion compounds of a host molecule with the same guest can occur if the host-guest interactions are flexible enough to allow nearly equienergetic, but crystallographically distinct host-guest packing arrangements. Reports of the preparations of such polymorphs are rare [163], but would probably increase in frequency if a variety of preparative conditions were to be employed.

As with most organic compounds, inclusion complexes are usually prepared only once and the chances of detecting polymorphism are correspondingly small. The well known host molecule cholic acid (7) has been shown to form different polymorphic inclusion complexes with the same guest [164]. The polymorph which crystallizes is determined by the absence or presence of a third chemical species. Crystallization of cholic acid from, e.g., acrylonitrile yielded the 1:1 host-guest complex with space group $P2_12_12_1$, while addition of acrylonitrile to a saturated solution of the host in butan-1-ol yielded a 1:1 cholic acid · acrylonitrile complex with space group $P2_1$. X-ray analyses confirmed that the polymorphs contained different packing arrangements as well as different hydrogen bonding networks. The controlling role of the third component in effecting precipitation of a specific polymorph was not clarified in the report but selective adsorption of solvent molecules on the surfaces of growing crystals may be involved.

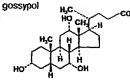
During the last ten years, considerable progress has been made in the control and modification of crystal properties using "tailor-made auxiliaries" [35, 36]. These auxiliaries may act either as promoters of crystal growth (making them useful for the study of nucleation processes) or as inhibitors (for use in the control of, e.g., morphology, enantiomeric resolution and polymorphism). The strategy for effecting kinetic resolution of a conglomerate involves the design of chiral-resolved polymeric inhibitors which will, in solution, bind selectively to only one of the growing enantiomeric crystalline phases, thus preventing its growth and permitting unhindered growth of the other enantiomeric phase. Where simultaneous crystallization of the racemic phase might occur, design of the inhibitor would have to take this into account. It must be emphasised that

successful design of auxiliaries depends on a detailed knowledge of the packing arrangements, as well as the relationship between packing arrangements and crystal morphology for all species that might appear in the system. An early demonstration of this approach (which was subsequently successfully applied to several other systems) involved resolution of racemic histidine hydrochloride [165]. In the report of this resolution, it was mentioned that this approach was being tested for use in the precipitation of metastable polymorphic crystals by kinetic control.



5 gossypol

6 indomethacin



7 cholic acid

Recent application of these principles to polymorphic control is shown schematically in Fig. 15 for a hypothetical dimorphic system in which one polymorph is centrosymmetric and the other crystallizes in a polar space group [36]. In the former crystal, the molecules are arranged in antiparallel orientation whereas in the latter they are aligned along a common direction. A tailor-made auxiliary which binds to both crystals would do so at the two (indistinguishable) ends of the centrosymmetric crystal but only at one end of the polar crystal. The latter would therefore grow at the expense of the former and polymorphic control will have been achieved.

This concept was successfully applied to *N*-(2-acetamido-4-nitrophenyl)pyrrolidene (PAN) where crystallization of the metastable polymorph of PAN could be induced by addition of a remarkably small amount (0.03%) of the designed inhibitor [36]. Generalisation of this strategy is not straightforward, however, since polar crystals may belong to space groups of relatively high symmetry which require the constituent molecules to adopt a variety of orientations with respect to the polar axis [35]. Strategies which have been explored to engineer non-centrosymmetry into crystals for NLO properties have been listed in a recent paper describing the polymorphism of 1,3-bis(*m*-nitrophenyl)urea [166].

Research on the use of tailor-made surfaces for promotion of nucleation has been pursued in parallel with studies of crystal growth inhibition [35, 36]. The objective here is to gain a deeper understanding of the mechanism of molecular

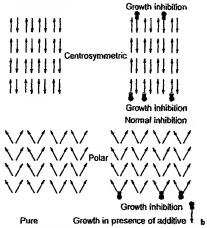


Fig. 15 a Schematic illustration of the packing arrangement in a polar and a centrosymmetric crystal. b The effect of a designed inhibitor on crystal growth. (Adapted from [36] with permission

clustering associated with the formation of pre-critical nuclei of crystalline phases. The relevance for organic crystal polymorphism is that direct observation of the dynamics of crystal nucleation could elucidate the effects of additives on polymorphic control. Much of this work has been based on the use of Langmuir films to induce nucleation at the air-water interface [36]. To illustrate the level of interpretation which is currently possible from such experiments, a recent study of the self-assembly of crystalline monolayers and multilayers of *n*-alkanes on a water surface is cited [167]. Surface observations were carried out using synchrotron grazing incidence X-ray diffraction and specular X-ray reflectivity. These allowed detailed characterisation of the crystalline films including determination of their space groups. It is noteworthy that these were found to be identical to those of the corresponding three-dimensional crystals, thus demonstrating that the full crystal symmetry already exists even for crystallites whose thickness corresponds to only a few molecular layers.

Related to the above strategy is the concept of engineering solid surfaces for promoting nucleation and epitaxial growth of desired polymorphs. If it is assumed that the pre-nucleation aggregate for a polymorph resembles the mature crystal, it should be possible to design a surface which mimics a particular crystal plane of the desired species, and upon which heterogeneous nucleation will occur and epitaxial polymorphic growth will result. This has been realised in the technique of ledge-directed epitaxy [168] in which the intersection of a terrace plane and a step on a crystal surface (a "ledge") functions as a nucleation site for organic crystals. For polymorphic control, the crucial requirement is that two well-defined, close-packed crystal planes of the polymorph have a dihedral

angle which matches that of the substrate ledge site. Successful application of this principle to the selective nucleation and growth of a thermodynamically less favoured polymorph of a complex organic salt on succinic acid single crystals as substrates has recently been reported [169].

The approaches outlined above continue to provide new insights into the fundamental process of crystallization of organic molecules and studies of the type described promise to contribute very significantly in the future to a detailed understanding of the molecular origins of crystal polymorphism and to its effective control. The author believes that the phenomenon of polymorphism will, notwithstanding such progress, retain its aura as a remarkable manifestation of Nature's diversity.

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EPSRC

Details of Grant

EPSRC Reference:	**EP/C002768/1**				
Title:	**Polymorph Selection and Control: The Generation of Hydrates and Solvates**				
Principal Investigator:	**Dr L Seton**				
Other Investigators:					
Researcher Co-investigator:					
Project Partner:					
Department:	**Pharmacy and Chemistry**				
Organisation:	**Liverpool John Moores University**				
Scheme:	**First Grant Scheme**				
Starts:	**09 May 2005**	Ends:	**08 November 2007**	Value (£):	**125,427**
EPSRC Research Topic Classifications:	**Chemical Structure** **Functional Organics and Polymers: Characterisation**				**Colloids, Soft Solids and Complex Fluids**
EPSRC Industrial Sector Classifications:	**Manufacturing** **Pharmaceuticals and Biotechnology**		**Chemicals**		
Related Grants:					
Panel History:	Panel Date Panel Name **21 Oct 2004 Chemistry Prioritisation Panel (Science)**				Outcome **Announced**

Summary

The manufacture of pharmaceutical materials and other fine chemicals involves purification, isolation and recovery by recrystallisation. Many drug substances exhibit polymorphism: the ability of a substance to exist in two or more solid forms with different arrangements of atoms or molecules. Different polymorphs of a drug can have different physical properties, which means they may act differently when introduced to the body. Hydrates have molecules of water incorporated into the crystal structure; solvates have molecules of solvent within the structure. This can also alter the physical properties, and consequently, hydrates and solvates are known as "pseudopolymorphs". It is common, in manufacturing processes, to induce nucleation by the addition of a second solvent in which the solute is less soluble (known as an anti-solvent, co-solvent or the drown out method). This research aims to develop the ability to select which polymorph or pseudopolymorph will be generated when crystallisation takes place using the drown out method. The experimental programme will involve the nucleation of drugs under different sets of conditions and determination of the factors that influence the generation of polymorphs and pseudopolymorphs. Crystal engineering techniques in which structurally similar molecules are introduced into the crystallisation mixture will be applied to control nucleation processes and selectively produce desirable solid forms.
The outcome of this research has several potential benefits:

1. If one polymorph has a desirable set of properties then producing this selectively can improve the ease of manufacture of the active drug substance and / or medicine.
2. More effective therapies can be developed.
3. Patent opportunities are potentially expanded.

Final Report Summary

The manufacture of pharmaceutical materials and other fine chemicals involves purification, isolation and recovery by recrystallisation. Many drug substances exhibit polymorphism: the ability of a substance to exist in two or more solid forms with different arrangements of atoms or molecules. Different polymorphs of a drug can have different physical properties, which means they may act differently when introduced to the body. Hydrates have molecules of water incorporated into the crystal structure. This can also alter the physical properties, and consequently, hydrates are known as "pseudopolymorphs". Being able to produce reliably the desired form of a drug is essential to a variety of industries, but especially the pharmaceutical industry.
This project has investigated the crystallisation of polymorphic and hydrate materials under a variety of conditions and using different methods. The effect of solvent environment has been examined by crystallisation from mixtures of different solvents and found to be effective at selecting polymorph and modifying crystal shape. The use of a drown out solvent (a second solvent in which the solute is less soluble) as employed in industrial processes has been investigated and for the amino acid glycine, been found to control the polymorph generated during crystallisation.
A methodology has been developed in which materials have been crystallised from ternary systems that form liquid crystal phases. A ternary system has three components: in this case the drug material, surfactant and solvent. When the three components are mixed, liquid crystal phases are produced. Liquid crystals are solution phases in which surfactant molecules arrange into semi-ordered structures. By changing the proportions of each of the three phases in the ternary mix, the type of liquid crystal structures can change. By carefully controlling the compositions of the mixture, and crystallising from different liquid crystal phases, it has been demonstrated that both the hydrated and the anhydrous forms of theophylline, a drug material, can be obtained selectively.
The outcome of this research has several potential benefits:
1. If one polymorph or solid form has a desirable set of properties then producing this selectively can improve the ease of manufacture of the active drug substance and / or medicine.
2. During drug development, all possible solid forms should be identified and characterised. This method provides a mechanism for identifying forms that may not appear during standard crystallisation.
3. Patent opportunities are potentially expanded.

Further Information:	
Organisation Website:	**http://www.livjm.ac.uk**

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